**AEROBIC BIODEGRADATION OF
ALTERNATIVE FUEL OXYGENATES IN
UNSATURATED SOIL COLUMNS**

THESIS

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AFIT/GEM/ENV/04M-13

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Abstract

Groundwater contamination problems caused by methyl-tertiary butyl ether (MTBE) in subsurface waters have prompted the search for a gasoline oxygenate replacement. In order to avoid the problems encountered with MTBE, it is prudent to evaluate the fate and transport in the subsurface of proposed replacements, such as ethanol. In this study, ethanol transport and degradation in unsaturated soil was investigated using a series of eight soil columns. This preliminary study was to see if the soil column system components functioned properly, how similarly the eight soil columns performed, and if soil oxygen concentration affected degradation of ethanol. Tracer tests, using sodium chloride, determined the hydraulic characteristics of the soil columns. Oxygen sensors measured microbial activity in the soil columns when ethanol was added to the columns. The sensors were part of a control system that stabilized oxygen concentration at two levels (8% in four columns and 16% in four columns) to see the effect of oxygen concentration on ethanol degradation. A gas chromatograph (GC) was used to quantify column influent and effluent ethanol concentrations.

The tracer tests showed an average retention time, pore volume, and mass balance error of 13.3 hr (+/- 1.4), 18.9 L (+/- 2.0), and 1.3% (+/- 3.8), respectively. The oxygen sensor data, which indicated a drop in oxygen concentration over time when ethanol was added, suggested that microbial activity was occurring. The microbial aerobic metabolism of ethanol caused the oxygen concentrations to drop to the set points of 8% and 16%, at which they stabilized. The GC analysis also showed ethanol degradation.

Influent ethanol concentrations were ~ 1000 ppm, column effluent concentrations were at or near the method detection limit (MDL) of 1 ppm for both oxygen concentrations.

The soil columns, constructed as part of this research, were demonstrated to be a good laboratory system that could be used to study aerobic degradation of ethanol in the vadose zone. Further research is required to test other fuel oxygenates to see if they will degrade in this system. Such oxygenate degradation studies will be critical in helping to find a safe alternative to MTBE.

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IN UNSATURATED SOIL COLUMNS

I. Introduction

Overview

This work broadens understanding of fuel oxygenate biodegradation under aerobic conditions in unsaturated soil columns. The fuel oxygenate ethyl alcohol was considered for this study. Ethyl alcohol is also known as ethanol and will hereby be referred to as such.

Background

Fuel oxygenation helps keep automobile engines running smooth, burning clean, and fuel efficient. The oxygenation of fuel eliminates the noisy, rackety sound that is the sign of a poorly running engine. This "knocking" occurs when unburned vapors explode spontaneously, resulting in one or more secondary detonations (in addition to the one created by the spark from the spark plug) instead of the one smooth, efficient charge that provides efficient engine operation. Knocking is a characteristic rattling or pinging sound from the engine. It reduces fuel economy, and in severe cases can cause engine damage (Jargon, 2003).

The first solution to knocking that scientists arrived at, in the early 1920s, was to add tetraethyl lead to gasoline. In the mid 1920s, tests were being conducted on the substance and several workers died from a form of sudden lead poisoning in which they became delirious and violent. These incidents lead to a push by public health reformers

to have leaded gas outlawed. However, there was no official federal body with the

powers to investigate manufacture and distribution of a new industrial product. By the

1960s, scientific evidence proved conclusively that airborne lead was a serious health

hazard. Efforts were renewed to outlaw lead in gasoline, with federal restrictions

governing the lead content of motor fuels effected in the 1970s. Lead exposure can cause

a wide range of illnesses in adults and poses especially high risks for children, affecting

neurological development, growth and intelligence (NRDC, 2003).

Since the late 1970s, methyl *tert*-butyl ether (MTBE) has been added to gasoline

in the United States to replace lead and other toxic components. MTBE acts both as an

octane enhancer and as an oxygenating compound, allowing both the elimination of

alkyl-lead antiknocking agents and reductions in automobile carbon monoxide emissions.

The 1990 Clean Air Act Amendments (CAAA) require that oxygenates be used in all

grades of gasoline to reduce vehicle emissions such as air toxics, carbon monoxide, and

volatile organic compounds (Peaff, 1994:8). As a result of the CAAA, MTBE (the most

common oxygenate) is currently added at concentrations of up to 15% to more than 30%

of all gasoline sold in the United States (USEPA, 1994:1).

The use of MTBE for gasoline oxygenation has introduced the chemical into

groundwater from spills and leaky underground storage tanks. MTBE is poorly adsorbed,

chemically and biologically stable, and very soluble in water, making it very mobile and

persistent in the environment (Liu *et al.*, 2001:2197). The long-term human health

effects of MTBE exposure are unclear. The U.S. Environmental Protection Agency

(EPA) has issued a draft drinking water lifetime advisory for MTBE of 20 to 200 µg/liter

(USEPA, 1995:1), a range of values which reflects the current uncertainty about the carcinogenicity of this compound (Hardison *et al.*, 1997:5601).

Research Problem

Because of the potential problems associated with the use of MTBE in gasoline, the focus of this study is to assess the fate of MTBE replacements. More specifically, the research problem is to develop a laboratory system capable of evaluating the fate of potential MTBE replacements in the vadose zone.

Research Objectives

The ultimate goal in this line of research is to use a soil column system to test whether alternative fuel oxygenates will aerobically biodegrade in the vadose zone and determine the extent of degradation, the degradation rates, and the byproducts produced from degradation. If it is successful, this aerobic degradation process could be used as a basis to design a remediation system for oxygenate contaminated groundwater (Deeb *et al.*, 2000:182).

The objectives of this study were to construct and operate a soil column system and to conduct an initial investigation of the influence oxygen concentration had on the degradation of the fuel oxygenate, ethanol, in a soil system.

Specific Research Questions

1. How do the hydraulic properties of the eight columns compare? How alike are they?

2. Is there evidence that biological activity is taking place in the soil columns?

3. Is there evidence that degradation of ethanol is taking place?

4. Did oxygen concentration have an influence on the degradation of ethanol?

Research Approach

Once the equipment was constructed and operating properly, an ethanol/tap water mixture was fed into the tops of the columns. The soil oxygen concentration was measured to determine if biological activity was taking place inside the soil columns. A tracer test was conducted to determine the hydraulic properties of the columns. A gas chromatograph (GC) analyzed influent and effluent samples to determine if degradation of the ethanol had occurred. Once it had been determined that degradation was occurring, the next step was to determine if oxygen concentration influenced the degradation of the ethanol.

Scope of Research

This research was limited in several ways. First, the only fuel oxygenate examined was ethanol. This decision was based on the fact that demonstrating the experimental system was working correctly was the highest priority. Ethanol is easily biodegradable and competent microbial populations can quickly establish themselves. Ethanol is already used as a fuel oxygenate and has similar chemical structure and characteristics as MTBE (these similarities are discussed in Chapter 2). To minimize the complexity of the data analysis and allow for sufficient replication (four replicates at each

oxygen content), soil oxygen concentration was the only variable tested (80% and 40% of saturation).

Definition of Terms

Aerobic Organism – An organism that uses oxygen as its terminal electron acceptor (Maier *et al.*, 2000:30).

Anaerobic Organism – The terminal electron acceptor is a combined form of oxygen metabolite or an oxidized metal (Maier *et al.*, 2000:30).

Biodegradation – The breakdown of organic compounds by microorganisms (Maier *et al.*, 2000:366).

BTEX – The primary concern with gasoline in the subsurface is groundwater contamination by the relatively mobile and toxic components of gasoline such as benzene, toluene, ethylbenzene, and xylene isomers, collectively referred to as BTEX (Goudar and Strevett, 1998:11). The use of ethanol as a formulating ingredient is increasing, making it likely to be encountered in groundwater plumes containing BTEX. A better understanding of its effects on BTEX bioremediation is warranted (Corseuil *et al.*, 1998).

Bulk Density – The weight of a material per unit of volume compared to the weight of the same volume of water.

Engine Knocking – Occurs when unburned vapors spontaneously explode before the flame reaches them. This results in one or more secondary detonations (in addition to the one created by the spark from the spark plug) instead of one smooth, efficient charge that provides the best mileage. Knocking creates a characteristic rattling or pinging sound from the engine. It reduces fuel economy, and in severe cases, can cause engine damage (Jargon, 2003).

Fuel Oxygenates – Primarily ethers and alcohols specifically added to gasoline to increase the octane rating, promote cleaner burning in gasoline engines, and/or improve other performance characteristics. The most common fuel oxygenates are methyl tertiary-butyl ether (MTBE) and ethanol. Other fuel oxygenates include: tertiary-amyl methyl ether (TAME), ethyl tertiary-butyl ether (ETBE), diisopropyl ether (DIPE), tertiary-amyl ethyl ether (TAEE), tertiary-butyl alcohol (TBA), tertiary-amyl alcohol (TAA), and methanol (USEPA, 2003).

Maximum Contaminant Level (MCL) – The highest level of a contaminant that is allowed in drinking water (USEPA, 2004c)

Methanogenic Microorganisms – Produce CH_4 by the fermentation of simple organic carbon compounds or oxidation of H_2 under anaerobic (without oxygen) conditions with the production of CO_2. Methanogenic conditions prevail in many contamination plumes after all other electron acceptors (O_2, NO_3, Fe^{+3}, and SO_4) have been used up by other members of the subsurface microbial community.

Natural Attenuation – The process by which indigenous microbial populations degrade pollutants within a natural environment (Maier *et al.*, 2000:235).

Octanol-Water Partition Coefficient (K_{OW}) – The octanol-water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. Octanol is an organic solvent used as a surrogate for natural organic matter. This parameter is used in many environmental studies to help predict the fate of chemicals in the environment. The octanol-water partition coefficient has been correlated to water solubility (USGS, 2004).

Pore Volume – The volume of water required to replace (flush out) water in a certain volume of saturated porous media. For example, if the total empty bed volume of a column is $V=10$ m^3 and the effective porosity of the column filled with soil is $n_e=0.4$, one pore volume (PV) equals 4 m^3 ($=n_e*V$) of water. Correspondingly, if 20 m^3 were flushed through the control volume, the porous media would have been exposed to 5 pore volumes of flushing. The pore volume concept is used either in an active pumping system (pump-and-treat) or in a natural attenuation scenario, where natural groundwater flow flushes the source zone. The equation for the number of pore volumes that pass through a control volume during a certain time t is

$$P_V=v_x t/L \qquad \text{or} \qquad L = v_x t/P_V \qquad \text{or} \qquad t = LP_V/v_x$$

where P_V is number of pore volumes (unitless), v_x is seepage velocity (m/d), L is length along flow path in control volume parallel to groundwater flow (m) and t is time (d) (Karvonen, 2004).

Retention time – The time it takes for one pore volume to pass through a control volume (i.e., pore volume/outlet flow = retention time) (USEPA, 2004b).

Terminal Electron Acceptor – During the oxidative process, electrons are removed from a substrate and passed via an electron transport chain to a terminal electron acceptor, in order to generate energy (Maier *et al.*, 2000:30).

Vadose Zone – Unsaturated (not completely filled with water) zone of soil lying between the earth's surface and the top of the ground water (the water table), in which pores within the geologic matrix are partially filled with air and partially filled with water. Also known as the unsaturated zone or the zone of aeration (Maier *et al.*, 2000:62).

II. Literature Review

Overview

This chapter reviews the history of fuel oxygenate use and outlines information on the use of MTBE and ethanol as gasoline additives, their health effects, relevant regulatory issues, and their occurrence and distribution in the environment. The factors affecting the negative impact of MTBE on the environment were also described. Current biodegradation perspectives for fuel oxygenates and soil column testing were discussed. Finally, this chapter concludes with a justification of why we need to better understand the fate and transport of gasoline fuel components in groundwater.

This chapter focuses on MTBE and ethanol because of the research problem stated in Chapter 1: MTBE has potentially negative environmental impacts and a replacement needs to be found. In some areas ethanol is used in place of MTBE. Because of this, ethanol is the model oxygenate in the soil column experiment for this research.

Background

History of Requirements for Fuel Oxygenates.

Governmental efforts to reduce exhaust emissions from automobiles have incited changes in gasoline formulation. Automobile use contributes to atmospheric contamination by gasoline components through volatilization and exhaust emissions (Keller *et al*., 1998; Calvert *et al*., 1993). The release of hydrocarbons, their partial

oxidation products, and associated nitrogen oxides (NO_x) contribute to the formation of ozone through photochemical oxidation reactions. Elevated concentrations of ozone can cause human health problems and crop damage. Additionally, the incomplete combustion of hydrocarbons in automobile engines results in the formation of carbon monoxide which has also been related to harmful human health effects. The U.S. government, in 1968, mandated emissions standards in an effort to reduce this pollution. The resulting use of catalytic converters significantly reduced emissions of hydrocarbons, nitrogen oxides (NOx), and carbon monoxide (Calvert *et al*., 1993); however additional strategies were needed due to the increase in the automobile industry.

The Clean Air Act Amendments of 1990 established the reformulated gasoline (RFG) program to help achieve carbon monoxide and ozone National Ambient Air Quality Standards in non-attainment areas (Moyer, 2003). The RFG program mandated that oxygenates be added to gasoline in these non-attainment areas; though selection of the specific oxygenate to be added was left to the petroleum refiners (Moyer, 2003). According to the United States Department of Energy (DOE), by the year 2002 over 50 million barrels of ethanol and over 74 million barrels of MTBE were produced in the U.S., with much of the MTBE being used as a gasoline oxygenate (DOE, 2002; Moyer, 2003).

In 1992 the winter oxygenated fuel program, mandatory in 40 U.S. metropolitan areas, required 2.7% oxygen by weight (15% MTBE or 7.3% ethanol by volume) to be added to gasoline (Moyer, 2003). Shortly thereafter, in 1995, Phase-one of the RFG program mandated year-round use of 2.0% oxygen by weight (11% MTBE or 5.4% ethanol by volume) in gasoline used in 28 metropolitan areas (Moyer, 2003). Phase-two

of the RFG program was initiated in 2000, maintaining the requirements established in Phase-one (Moyer, 2003). However, because of the widespread use of oxygenated fuels, one cannot be assured of the oxygenate status of gasoline that is sold, distributed, or leaking in any particular region of the country (USEPA, 1998c).

California has led the way in developing regulations related to MTBE. Because MTBE has been detected in groundwater and surface water sources throughout California, the governor of California issued an executive order in March 1999 to ban MTBE in the state's gasoline by the end of 2002 (ACWA, 2003). This date was later moved to 2004 due to logistical problems experienced by oil companies. The ACWA (2003) also noted that MTBE contamination has forced the closure of drinking water wells in South Lake Tahoe, Santa Monica, San Jose, Cambria, Kern County and other locations. It has resulted in millions of dollars in water treatment, cleanup and replacement water costs, and has diminished the public's confidence in the safety of water supplies.

Classes of Oxygenates.

Two classes of oxygenates, alcohols and ethers, may be found in gasoline. These classes differ in water solubility and mobility. Alcohols are a broad class of organic compounds containing a hydroxyl (-OH) functional group (Figure 1). Alcohols can be made from plant matter or synthesized from petroleum derivatives. They are used in organic synthesis as solvents, in the manufacturing of detergents, in pharmaceuticals, foods, plasticizers, and fuels.

Methanol   Ethanol
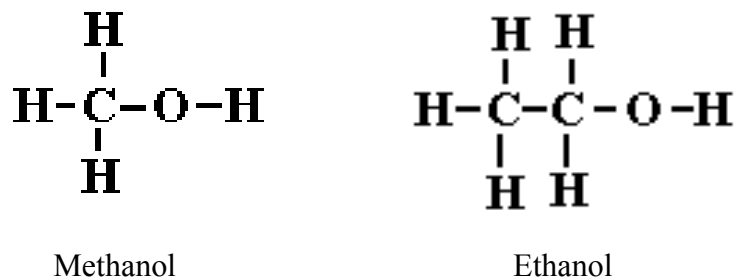
Figure 1. Structural formula of alcohols

Ethers are a class of organic compounds in which an oxygen atom is interposed

between two carbon atoms: C-O-C (Figure 2). Ethers are manufactured from petroleum

derivatives and are widely used as industrial solvents.

a) Ethyl tert-butyl ether   b) Methyl tert-butyl ether   c) Tert-amyl methyl ether
(ETBE)   (MTBE)   (TAME)

d) Tert-butyl alcohol   e) Tert-butyl formate   f) Tert-amyl alcohol
(TBA)   (TBF)   (TAA)
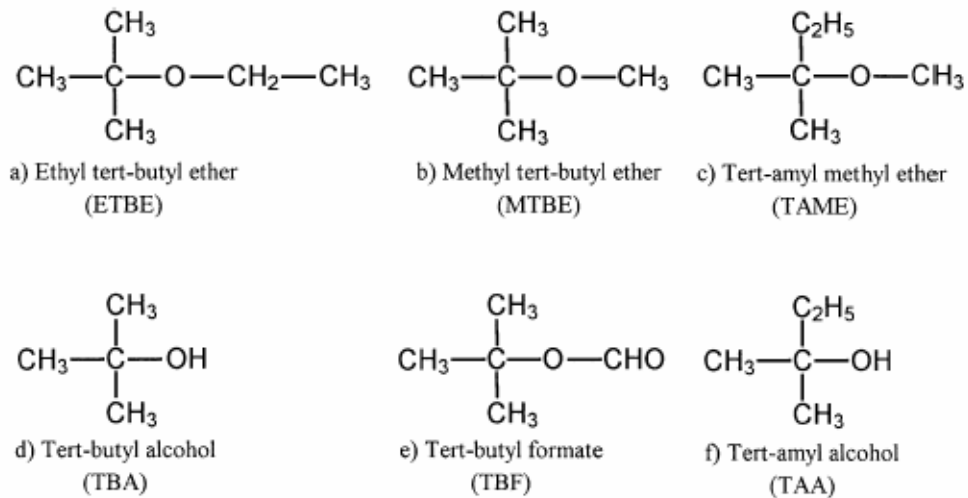
Figure 2. Structural formula of specific oxygenates (a, b, c) and their major metabolic
intermediates (d, e, f) (Kharoune *et al.*, 2001:1667)

Extent of MTBE and Ethanol Use.

Gasoline and similar fuels are derived from petroleum and are composed

primarily of hydrocarbons (compounds containing only carbon and hydrogen atoms).

Oxygenates contain oxygen atoms in addition to carbon and hydrogen atoms and are synthesized from petroleum derivatives or plant matter. Oxygenates are added in relatively large concentrations (>5%) and are considered blend components of gasoline (USEPA, 1998c:1). Oxygenates can be added as high purity chemicals, or as technical grade chemicals with traces of other ethers and alcohols. MTBE and ethanol were introduced as gasoline additives in 1979 and are currently the most frequently used gasoline oxygenates (USEPA, 1998c).

MTBE.

MTBE is a synthetic chemical mixed with gasoline for use in reformulated gasoline (RFG) (USEPA, 1998a:1). It is a liquid, generally made by combining isobutylene and methanol (USEPA, 1998a:1). MTBE was developed in the 1940s; but was not commercially produced until the 1970s (USEPA, 1998a:1). MTBE was used commercially for the first time in Europe as a gasoline blending component. It was first introduced in the 1980s in the United States as an octane booster to replace alkyl lead additives.

MTBE is also added to gasoline in areas that currently do not require the use of RFG (Moyer, 2003). Although added in lower quantities than in RFG, MTBE is added to premium gasoline, as well as regular gasoline in lower proportions, for its octane boosting properties (Moyer, 2003). It is estimated that MTBE is present in 30 to 50 percent of all gasoline sold in the United States (OFA, 2003).

MTBE is regulated under several laws, including the CAA; the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA); and the

Emergency Planning and Community Right-to-Know Act. Several agencies also have

responsibilities for regulating MTBE, including the EPA Offices of Water, Solid Waste,

and Emergency and Remedial Response, as well as the Consumer Product Safety

Commission (USEPA, 1998a:2; Rhodes and Verstuyft, 2001:2).

Ethanol.

Ethanol is an alcohol made from renewable resources such as corn and other

cereal grains, food and other beverage wastes, and forestry by-products (ICGA, 2004).

Ethanol-blended fuel substantially reduces carbon monoxide and volatile organic

compound emissions, which are precursors to ozone. The corn-based substance is added

to gasoline blends to meet oxygenate level requirements mandated by the 1990 Clean Air

Act Amendments and to raise the octane level.

Ethanol adds oxygen to gasoline like MTBE, however, there are no health or

environmental concerns associated with ethanol use. Ethanol is an organic, non-toxic

substance that is even consumable by humans - the ethanol that is in alcoholic beverages

is the same alcohol that is added to gasoline (Armstrong, 1999). In fact, adding ethanol

to gasoline reduces the amount of toxic substances in the fuel we burn (ICGA, 2004;

CFDC, 2003).

Ethanol has been used in gasoline since the early 1900's. Ethanol was used as a

fuel extender during both World War I and World War II (CFDC, 2003). This was done

because petroleum based fuels were needed on the war front. After both wars, ethanol

use declined sharply, largely because of its higher cost compared to gasoline. Ethanol

again came into favor as a fuel extender during the Arab Oil Embargo of 1973 and also during the early 1980's when oil prices again skyrocketed (CFDC, 2003).

Ethanol is used in approximately 15% of the oxygenated fuels (USEPA, 1998c). However, ethanol is generally used in the winter months since it increases the vapor pressure of gasoline thereby increasing gasoline volatility. Ethanol is used as a gasoline oxygenate in other countries. In Brazil, approximately 85% of the automobiles use gasoline containing 22 to 24% ethanol. The remaining automobiles use hydrated ethanol for fuel (Corseuil *et al.*, 1998).

The Production and Distribution of MTBE and Ethanol.

Ethanol is produced mostly from microbial fermentation of corn with a small percentage produced from chemical syntheses techniques (ICGA, 2004). MTBE is produced from isobutylene at refineries and at chemical companies from either butane or isobutane as raw material (Morse, 1999). The total production of ethanol (Pimental, 1998) and MTBE (Morse, 1999) in 1998 was approximately 1 billion and 3.1 billion gallons, respectively. Ethanol's tendency to separate from gasoline and solubilize water into ethanol blended gasoline, makes it necessary to introduce ethanol into gasoline at or near the distribution terminals (USEPA, 1998c). However, MTBE is generally blended with gasoline at refineries and distributed by pipeline.

Health Effects of Ethanol, MTBE, and Other Gasoline Components.

The prevalence of petroleum hydrocarbon releases from oil production sites, underground storage tank sites (USTs), and refineries is one of the most important environmental issues that our nation faces. Chemicals of concern at these sites include

benzene, toluene, ethyl benzene, xylenes (BTEX), total petroleum hydrocarbons (TPH), lead, MTBE, and MTBE metabolites (tert-butyl alcohol (TBA) and formaldehyde). Of these constituents, benzene has been demonstrated to be a human carcinogen and the others all pose health risks (Corseuil *et al*., 1998). Due to the toxic nature of the chemicals released and the fact that many of these sites are located near residential properties and drinking water sources, potential impact to human health is high.

Inhalation and the ingestion of contaminated groundwater are the possible methods of human exposure to ethanol as a fuel oxygenate. The sources of ethanol in the air that contribute to exposure by means of inhalation include: refueling activities, exhaust emissions, and evaporative emissions. Research on levels of ethanol in the blood of mice following exposure to several doses of inhaled ethanol suggested that the levels of ethanol likely to be inhaled during typical refueling would not result in toxic effects to humans (Pastino *et al*., 1997).

The health impacts of MTBE on humans are not completely understood; however, many studies have been conducted on laboratory animals and even some on human volunteers (Williams and Sheehan, 2003). Because of this uncertainty, the USEPA has yet to establish a maximum contaminant level (MCL) for MTBE.

Results from sub-chronic animal studies indicate that the most vulnerable organs to exposure by MTBE are the kidney and liver (Williams and Sheehan, 2003). Increased kidney weights, cell proliferation, and kidney lesions have been observed in several studies (Williams and Sheehan, 2003). The sub-chronic effects of MTBE are similar for both ingestion of MTBE-contaminated water and inhalation of MTBE vapors. Other reported effects include reversible nervous system ailments (Williams and Sheehan,

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2003). Exposure to MTBE has not resulted in any observed adverse effects to reproductive health of laboratory animals (Williams and Sheehan, 2003). The reported effects of TBA exposure are similar to those of MTBE exposure. Human studies investigating inhalation and ingestion of MTBE indicated limited short-term adverse respiratory and neurological effects; however, there are no specific long-term data available for exposure to MTBE or TBA (Williams and Sheehan, 2003).

The USEPA refers to the threshold value for ingestion as the Reference Dose (RfD), and the threshold airborne concentration for inhalation as the Reference Concentration (RfC) (Williams and Sheehan, 2003). The reference dosage or concentration corresponds to a level of exposure below which no negative health effects should be observed, which is similar to the no-observed-adverse-effect level (NOAEL). The EPA has yet to establish an RfD for MTBE ingestion; however, the EPA has established an RfC for MTBE inhalation. The RfC for MTBE exposure has been established at 3 mg/m^3 (USEPA, 2003). Williams and Sheehan (2003) point out that extrapolation of the RfC is appropriate for determination of the RfD and this extrapolation corresponds to an RfD of approximately 1 mg/kg/day (Williams and Sheehan, 2003). Comparison of threshold values compiled in Williams and Sheehan (2003) indicate that the allowable MTBE concentrations are on the order of 10 times higher than other gasoline constituents such as BTEX (Williams and Sheehan, 2003). This suggests that, in general, MTBE has a much lower non-cancer toxicity than BTEX.

Laboratory studies on the carcinogenicity of MTBE indicate that MTBE does pose a cancer threat to animals (Williams and Sheehan, 2003). The EPA has recognized MTBE as an animal carcinogen but has not officially declared that it is a potential cancer

risk to humans (Williams and Sheehan, 2003). The MTBE metabolites, TBA and formaldehyde, also showed marginal evidence of posing a cancer threat to animals (USEPA, 1997). Some states have established drinking water standards based on the assumption that MTBE does in fact pose a cancer risk to humans (Williams and Sheehan, 2003).

The USEPA has suggested, based on taste and odor concerns, a drinking water advisory level of 20-40 µg/L for MTBE (USEPA, 1998a:2; Liu *et al.*, 2001:2197; Rosell *et al.*, 2003:172). Although strictly based on aesthetic considerations, the drinking water advisory levels are considered protective of health since they are 20,000 to 100,000 times lower than reported adverse exposure levels (USEPA, 1997). States have established MCLs notwithstanding the lack of guidance from the EPA. California's Department of Health Services (DOHS) has developed, based on taste and odor concerns, an enforceable secondary drinking water maximum contaminant level (MCL) of 5 µg/L for MTBE (Rhodes and Verstuyft, 2001) and Texas established an MCL of 240 µg/L, the highest of any state (Williams and Sheehan, 2003). Other states have established action levels ranging from 10 to 202,000 µg/L (Williams and Sheehan, 2003).

Release of Gasoline Oxygenates into the Environment.

The release of fuel oxygenates into the atmosphere, surface waters, and groundwaters is due to their production, distribution, storage and use. Oxygenate release into the atmosphere is quantitatively the largest reported release mechanism (Zogorski *et al.*, 1997), but groundwater contamination, especially by MTBE, is currently the major concern. Examples of subsurface contamination sources are pipelines, refueling

facilities, surface spills, precipitation, and especially underground storage tanks (USTs).

There are millions of oil production sites, USTs, and refineries located throughout the

United States alone. However, the decrease in the number of USTs and the improvement

of their structure as mandated by the United States Environmental Protection Agency

(USEPA) and state requirements should reduce the number of leaking USTs. In spite of

this, in 1995 an estimated 200,000 UST sites still required funding for investigation or

cleanup (Gurr and Homann, 1996). MTBE has been detected in approximately one half of

the groundwaters associated with leaking USTs in California (Keller *et al.*, 1998). The

increased use of ethanol in Brazil has raised concerns and initiated scientific research on

the impact of ethanol on the fate and transport of hydrocarbons in subsurface gasoline

spills (Corseuil *et al.*, 1998; Corseuil, 1999).

The MTBE Problem

MTBE has been detected in groundwaters throughout the U.S. This has driven

research to evaluate the environmental behavior and potential health impacts associated

with the use of MTBE as a gasoline oxygenate. A better understanding of the occurrence

and persistence of MTBE in the environment (especially in groundwater) will provide

important information necessary for predicting the fate of other gasoline oxygenates, of

which there is limited scientific information regarding their environmental behavior as

gasoline oxygenates. For this reason, this section discusses the current state of

knowledge related to the fate and transport of MTBE in the environment.

Occurrence and Distribution of MTBE in the Environment.

The extent of MTBE usage, along with its persistence (i.e. lack of biodegradability, which will be discussed in the next section) and mobility in the environment, contribute to making MTBE a common volatile organic chemical detected in many groundwater sources. The sources of MTBE are widespread including fuel leaks and spills, engine emissions, precipitation, and run-off. Additionally, MTBE sources can be difficult to identify. The broad spectrum of sources coupled with the separation of the BTEX-plume from the MTBE-plume may cause significant uncertainty as to the actual source of MTBE contamination in any particular instance (Squillace *et al.*, 1996).

Groundwater samples were taken from 210 wells in urban areas and 549 wells in agricultural areas across the US during a period from 1993 to 1994 as part of the US Geological Survey National Water-Quality Assessment program (Squillace *et al.*, 1996). MTBE was the second most common volatile organic chemical detected (Squillace *et al.*, 1996). Of the urban wells sampled, 27% contained MTBE and of the agricultural area wells sampled, only 1.3% contained MTBE (Squillace *et al.*, 1996). Squillace *et al.* (1996) suggest that leaking underground storage tanks are most likely the primary source of MTBE releases into the subsurface.

The DoD is also responsible for MTBE releases throughout the country. According to the Air Force Center for Environmental Excellence (AFCEE) Environmental Resources Program Information Management System (ERPIMS) database at least 40 Air Force installations have reported detections of MTBE contamination in groundwater.

Table 1 summarizes the Air Force installation, source, and magnitude of concentrations of MTBE in groundwater reported.

Table 1. Summary of USAF MTBE-Contaminated Sites (AFCEE, 2003)

Installation	Sample Site	Maximum Reported MTBE Conc. (µg/L)
Goodfellow AFB, TX	Drum Storage Area	60,400
Andrews AFB, MD	Main Service Station	60,000
Lackland AFB, TX	UST	34,800
Randolph AFB, TX	BX Service Station	21,000
Vandenberg AFB, CA	BX Service Station	11,000
March AFB, CA	N/A	5,500
Travis AFB, CA	North and South Gas Station	5,400
Moody AFB, GA	BX Service Station	3,400
Griffiss AFB, NY	Apron 2	3180
Nellis AFB, NV	Maint. Fac. (TCE-plume)	1,700
Avon Park AF Range, FL	10,000 gal AST	1,500
Tinker AFB, OK	UST, Site 23	1,200
Seymour-Johnson AFB, NC	BX Service Station	690
Plattsburgh AFB, NY	N/A	529
McConnell AFB, KS	N/A	420
Carswell AFB, TX	Base Service Station	330
George AFB, CA	N/A	327
Dover AFB, DE	Tank Farm	260
Chanute AFB, IL	N/A	248
Loring AFB, ME	N/A	190
Williams AFB, AZ	N/A	139
Maxwell AFB, AL	UST	123
Holloman AFB, NM	Military Gasoline Station	120
MA Military Reservation	Residential Wells	73
Patrick AFB, FL	ST-28 Area	59
Keesler AFB, MS	N/A	56
Scott AFB, IL	Military Gasoline Station	56
Charleston AFB, SC	Base Gasoline Station Leak	48.1
Pope AFB, NC	N/A	38
Eglin AFB, FL	Gasoline Dispensing Facility	27.3
Brooks AFB, TX	Fire Protection Training Area	25
Laughlin AFB, TX	Fire Protection Training Area	24
Beale AFB, CA	Test Cell Discharge Area	20.7
Little Rock AFB, AK	Fuel Spill	19

Installation	Sample Site	Maximum Reported MTBE Conc. (µg/L)
F. E. Warren AFB, WY	Gasoline Spill Site	12.3
Pease AFB, NH	N/A	12
Johnston Island	JP-5 AST	11.4
Offutt AFB, NE	Fire Protection Training Area	11
Tyndall AFB, FL	N/A	9.4
Hickam POL Facility, HI	Fuel Line Leak	2.2
Wurthsmith AFB, MI	Fuel Spill Site	2.1
Myrtle Beach AFB, SC	Gasoline Storage Tank	1.4
Hurlburt Field, FL	UST Leak	1.3
McClellan AFB, CA	N/A	1

Biodegradation of MTBE and Other Ether Oxygenates.

The resistance of MTBE to complete mineralization (oxidation to carbon dioxide and water) by microorganisms has been reported under both aerobic (Barker *et al*., 1990) and anaerobic conditions (Suflita and Mormile, 1993; Mormile *et al*., 1994; and Yeh and Novak, 1995). The results of Suflita and Mormile's work shown in Table 2, indicate that the majority of ether oxygenates containing tertiary or quaternary branching, including MTBE were not biodegraded in anaerobic sediment slurries. Similarly, tert-butyl alcohol (TBA) (an MTBE biodegradation intermediate), is also resistant to microbial attack (Novak, *et al*. 1985; Suflita and Mormile, 1993; Mormile *et al.*, 1994) and has been detected in groundwaters impacted by MTBE (Landmeyer *et al*., 1998). In contrast, ethanol is a straight-chain alcohol and was rapidly biodegraded in the anaerobic sediment slurries (Table 2). Straight-chain alcohols, ketones, esters, and the straight-chain analog of MTBE, methyl butyl ether, have been found to be biodegradable under a variety of anaerobic conditions (Mormile *et al*., 1994). Because biodegradability typically

decreases with increased chemical branching, highly branched oxygenated organic

compounds, including MTBE, have a longer residence time in the environment.

Table 2. Rates of Anaerobic Biodegradation of Several Gasoline Oxygenates in Aquifer Slurries (Suflita and Mormile, 1993)

Oxygenate	Rate (ppm C-day^{-1})		Oxygenate	Rate (ppm C-day-1)
Alcohols			**Ethers**	
Methanol	7.4		Methyl tert-butyl ether	0
Ethanol	17.9		Methyl tert-amyl ether	0
2-proponol	7.6		Ethyl tert-butyl ether	0
tert-butanol	0		Isopropyl ehter	0
			Diethyl ether	0
Esters			Propyl ether	0
ethyl acetate	16.6		Butyl ether	0
Ethyl acetate	13.7		Butyl methyl ether	0.5
Methyl propionate	7.3		Butyl ethyl ether	0
Methyl isobutyrate	4.1		**Ketones**	
			Methyl ethyl ketone	9.4
			Acetone	7.3
			Methyl isobutyl ketone	21-28

Fate and Transport of Oxygenates in the Environment

MTBE detection in ground and surface waters suggests that unfavorable

consequences can be anticipated if chemicals that resist biodegradation are added to

gasoline. The ability of gasoline additives, including MTBE and ethanol, to

biodegradation is an important characteristic in evaluating the fate of gasoline oxygenates

in fuels. In addition, an understanding of the fate and transport of oxygenated fuels

requires information on the behavior of contaminant mixtures (Brusseau *et al.*, 1991).

High concentrations of ethanol are likely to be found in groundwater contacting

non-aqueous phase ethanol-blended gasoline because oxygenated gasoline can contain

high concentrations of ethanol, which is infinitely soluble in water (Table 3). Due to the

low solubility of other gasoline components including the BTEX hydrocarbons relative to

ethanol (Table 3), ethanol would likely be the dominant dissolved component near the

source areas.

Table 3. Summary of Chemical Properties of Alcohols, Ethers, and BTEX
Compounds (USEPA, 2004a; Moyer, 2003)

Alcohols	Molecular Weight (g/mol)	Pure Phase Water Solubility (mg/L)	log K_{ow} (log l/kg)	Vapor Pressure (mm Hg)	Henry's Law Constant (dimensionless)	Specific Gravity
Gasoline	~100	100-200				0.72-0.74
Ethanol	46.069	miscible		49 - 56.5	0.00021 - 0.00026	0.789
MTBE	88.149	43,000 - 54,300	0.94-1.30	245 - 256	0.023 - 0.12	0.741
Benzene	78.11	1780	1.56-2.15	95.19	0.222	0.88
Toluene	92.13	535	2.11-2.8	28.4	0.243	0.87

The octanol-water partition coefficient (K_{ow}) is the ratio of the concentration of a

chemical in octanol and in water at equilibrium and at a specified temperature. The low

K_{ow} values indicate that the ethanol and MTBE do not partition well to other organic

matrices. The result of the differences in adsorption is that the ethanol or MTBE plume

eventually outpaces and separates from the BTEX compounds that sorb more readily to

aquifer solids. The low Henry's constant of ethanol and MTBE indicates that they are

not as volatile as the BTEX compounds from the dissolved phase. The vapor pressure

indicates that MTBE is much more volatile than ethanol and the BTEX compounds from

the pure phase. As a result of these properties, some remediation technologies, such as

vapor extraction and granular carbon adsorption, are not as effective for MTBE as they may be for BTEX compounds. Figure 3 graphically depicts the relative differences in important chemical properties of several gasoline constituents.
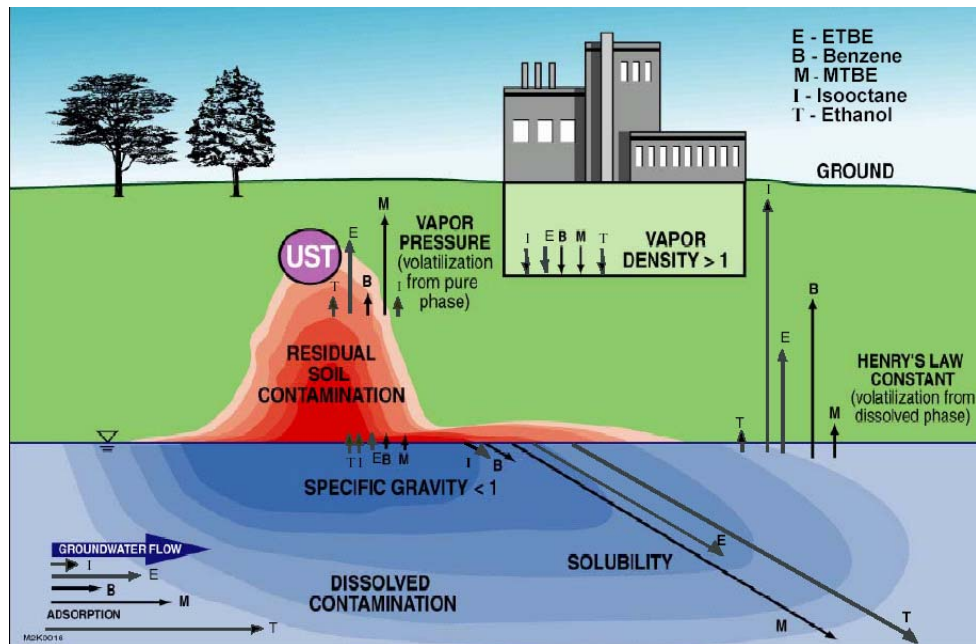
Figure 3. Graphical representation of chemical properties of several gasoline constituents and oxygenates (Jansen *et al.*, 2002)

Based on the chemical behavior of ethanol it is expected that ethanol in subsurface oxygenated gasoline spills will rapidly partition into groundwater and become the dominant dissolved contaminant immediately downgradient of the spill. The abiotic mechanisms for the attenuation of subsurface contaminants including sorption, volatilization, and abiotic degradation will not contribute substantially to the decreased mobility or loss of ethanol in subsurface aquifers. Therefore, the fate and transport of ethanol in groundwater aquifers will primarily be controlled by biodegradation.

Biodegradation of Ethanol and MTBE

The purpose of this section is to give a brief overview of some of the biological processes capable of degrading ethanol and MTBE to innocuous end-products. The evaluation of oxygenate biodegradation is necessary for predicting the fate and transport of oxygenates in the environment. This includes an understanding of the occurrence of ethanol-utilizing bacteria, the metabolic pathways and intermediates involved, the rates of biodegradation under diverse environmental conditions, and the factors that may govern biodegradation and the intermediates formed from degradation. An important factor that governs the biodegradation of contaminants is the electron-accepting status of the environment. While ethanol is relatively easily degraded under aerobic and anaerobic conditions, the rates and metabolic pathways of ethanol oxidation are clearly impacted by the electron accepting conditions.

Early biological degradation studies done on MTBE indicated little or no degradation and very low to negligible cellular yields; consequently many considered MTBE recalcitrant to biological degradation processes. Since the publication of these studies, more recent studies have shown that MTBE is in fact susceptible to biological degradation by pure and mixed cultures as well as at least one species of fungus.

Aerobic Biodegradation.

Ethanol.

Organic substrates that can easily be converted to compounds that enter central metabolic pathways of bacteria are generally rapidly biodegraded. In this regard, after a limited number of metabolic reactions, ethanol is converted to acetyl coenzyme A which

enters the tricarboxylic acid cycle (TCA), the primary energy-generating pathway in aerobic metabolism (McKinney and Jeris, 1954). Thus, due to the relative ease with which ethanol enters the TCA cycle, ethanol is rapidly metabolized by aerobic microorganisms (McKinney and Jeris, 1954). Further, the enzymes necessary for incorporating ethanol into the TCA cycle (i.e. ethanol and acetaldehyde dehydrogenase) are widely distributed among microorganisms (McKinney and Jeris, 1954).

The prevalence of aerobic microorganisms capable of degrading ethanol was demonstrated in laboratory screening exercises that identified 363 strains of bacteria capable of growing on 1.5% ethanol (Okumura, 1975). Several of these strains are known soil inhabitants suggesting that these findings have environmental relevance (Okumura, 1975). Ethanol has been shown to rapidly degrade in aerobic sewage sludge (McKinney and Jeris, 1954) and in aerobic subsurface sediments (Corseuil et al., 1998).

MTBE.

Numerous laboratory and field studies have reported the biodegradation of MTBE and other fuel oxygenates under aerobic conditions. Steffan et al. (1997) evaluated the ability of propane-oxidizing bacteria to metabolize gasoline oxygenates, including MTBE, ETBE, and TAME. Hardison et al. (1997) studied the degradation of diethyl ether (DEE) and MTBE by Graphium sp. Both of these studies suggested that the oxidation enzyme responsible for MTBE biodegradation may be a cytochrome P450 enzyme.

Kharoune et al. (2001) evaluated the feasibility of continuous aerobic biodegradation of a mixture of the oxygenates ETBE, MTBE and TAME, in an upflow

fixed-bed reactor (UFBR). The results of this study showed a higher resistance to biodegradation exhibited by MTBE and TAME than that of ETBE. Kharoune *et al*. (2001) suggested that the attacking enzyme is not able to react with MTBE and TAME, due to the arrangement of atoms; and that the major limiting step to aerobic degradation of the oxygenates may be the ease with which the ether bond is broken, but not the accumulation of intermediates.

Another laboratory study evaluated the effect of oxygen supply on MTBE degradation (Yang *et al.*, 1998). This study showed that a continuous supply of oxygen, compared to a one-time oxygen addition, greatly enhanced MTBE degradation rates.

Anaerobic Biodegradation.

Ethanol.

The decay of organic matter in anaerobic environments occurs by microbial consortia that can be viewed as several physiological groups of microorganisms operating at different points in the anaerobic food chain (Maier *et al*., 2000:368). The first group, fermentative bacteria, degrade polysaccharides, proteins, and lipids with the production of organic acids, alcohols, H_2, and CO_2. Hydrogen gas, a variety of alcohols, and organic acids are, in turn, utilized in anaerobic respiration with a variety of alternate electron acceptors including manganese oxides, ferric oxides, nitrate, and sulfate (Maier *et al*., 2000:368). In the absence of alternate electron acceptors, the biodegradation of ethanol and many of the organic acids is catalyzed by syntrophic bacteria to acetic acid and H_2 (McInerney and Bryant, 1981). Methanogenic bacteria catalyze the transformation of H_2 and acetic acid to methane and carbon dioxide (McInerney and Bryant, 1981).

Methanogenic bacteria and microorganisms utilizing alternate electron acceptors (including sulfate-reducing bacteria, nitrate-reducing bacteria, and iron-reducing bacteria) are considered terminal members of the anaerobic food chain because they typically oxidize substrates to gaseous end products (Maier *et al*. 2000).

The common occurrence of ethanol in anoxic environments is attributed to the fact that ethanol is produced during the fermentation of a variety of compounds distributed among both aquatic and terrestrial plants. Ethanol-producing bacteria have been isolated from soil, sewage sludge, estuarine sediments, decaying grass, and decaying trees (Jayasekera *et al*, 1989). Interestingly, plants are also known to metabolize ethanol and incorporate the carbon from ethanol into plant tissues (Jayasekera *et al*, 1989).

Despite the importance of ethanol as a fermentation intermediate, it is detected at very low concentrations in the environment indicating that rapid anaerobic ethanol metabolism occurs, thereby preventing its accumulation in-situ (ICGA, 2004).

MTBE.

Studies have shown different results for the anaerobic biodegradation of MTBE. The soils of three different sites, under various anaerobic and anoxic conditions, were evaluated by Yeh and Novak (1995). They found that the biodegradation of MTBE was observed under methanogenic conditions in a soil with a low organic carbon content. It was shown that easily degraded organic compounds actually inhibited MTBE degradation. However, microcosm studies (Bradley *et al.*, 2001; Finneran and Lovley, 2001; and Somsamak *et al.*, 2001) of aquifer and surface water sediments have

demonstrated the capability of indigenous bacteria to degrade MTBE to carbon dioxide and/or methane under various terminal electron acceptor conditions. While there have been few successful laboratory or field experiments showing MTBE biodegradation under anaerobic conditions, there is some evidence that MTBE is reduced under methanogenic conditions in the field (Stocking *et al.*, 2000:188).

Biodegradation of Hydrocarbons in Contaminated Aquifers.

The biodegradation of hydrocarbons by indigenous subsurface bacteria is the primary mechanism for the natural attenuation of fuel spills in aquifers (Salanitro *et al.*, 1997). Aerobic microorganisms have the physiological capacity to oxidize the majority of hydrocarbons including BTEX, which are of increased concern due to their high aqueous solubility and toxicity. Aerobic degradation does play an important role in the removal of BTEX from groundwater naturally (Salanitro, 1993), but the available oxygen reserves are usually rapidly depleted once a site is contaminated with gasoline hydrocarbons. Biodegradation in the resulting anaerobic environments is dependent on the availability of alternate electron acceptors including solid-phase manganese and ferric oxides along with soluble electron acceptors including nitrate and sulfate (Corseuil, 1998). In subsurface aquifers contaminated with petroleum products or landfill leachate, the availability of electron acceptors decreases with distances towards the source of contamination. This results in the zonation of terminal electron accepting processes (Baedecker *et al.*, 1993; Lyngkilde and Christensen, 1992). Oxygen is generally more available near the leading fringe (farthest from source) of a hydrocarbon plume where the more soluble hydrocarbons (ie. BTEX) are the dominant contaminants. Oxygen quickly

becomes depleted moving towards the source area where anaerobic respiratory processes supported by nitrate, ferric iron, and sulfate, are dominant. Methanogenesis is most important adjacent to the source area where electron acceptors are exhausted due to the increased contaminant load and the increased time in which the source areas have been contaminated.

Anaerobic microorganisms have the capacity to oxidize a many different petroleum hydrocarbons including BTEX (Krumholz *et al*., 1996), alkanes (Reuter *et al*., 1994; Caldwell *et al*., 1998), and polycyclic aromatic hydrocarbons (Coates *et al*., 1996). The involvement of anaerobic processes towards the degradation of hydrocarbons in contaminated aquifers has been thoroughly studied (Barbaro *et al*., 1992; Chapelle *et al*., 1996; Lovley, 1997; Gieg *et al*., 1999). Thus, it is primarily through the activity of both aerobic and anaerobic microorganisms that hydrocarbon plumes eventually stabilize and shrink in size. It is therefore important to consider the influence that ethanol may have on the biodegradation of hydrocarbons.

Influence of Ethanol on Aerobic and Anaerobic BTEX Biodegradation.

The mechanism by which ethanol is most likely to impact BTEX plumes is its potential impact on biodegradation. This is because ethanol does not affect the abiotic factors that govern the transport of monoaromatic hydrocarbons. The available research regarding the effects of ethanol on the biodegradation of hydrocarbons is limited. The majority of this research has been conducted in Brazil where 85% of the cars run on gasoline containing 22% ethanol. Experiments conducted using slurries of sediment with no previous exposure to gasoline hydrocarbons revealed both inhibitory and stimulatory

effects on BTEX biodegradation, depending on the electron accepting conditions tested. Under aerobic conditions, BTEX was not degraded until ethanol was biodegraded to low levels, apparently due to the preferential utilization of ethanol (Corseuil *et al*, 1998; Hunt *et al*., 1997). However, additional studies demonstrated that *Pseudomonas putida*, a well studied aerobic hydrocarbon-degrading microorganism, degraded benzene, toluene, and ethanol simultaneously under aerobic conditions (Hunt et al., 1997). This finding demonstrates that additional research is needed before the effects of ethanol on aerobic BTEX biodegradation can be fully understood. Nevertheless, because large quantities of ethanol are released from spills of ethanol-blended gasoline, ethanol may deplete the available oxygen thereby limiting the aerobic biodegradation of BTEX. Since anoxic conditions will likely prevail in aquifers impacted with ethanol, the impact of ethanol on anaerobic BTEX biodegradation deserves consideration.

The only BTEX hydrocarbon found to degrade anaerobically in the experiments of Corseuil *et al* (1998) was toluene, which depended on the electron-accepting conditions.   When ethanol biodegradation did not deplete the available nitrate, ethanol did not affect the rate or extent of toluene biodegradation under nitrate-reducing conditions. The presence of ethanol decreased toluene biodegradation in iron-reducing and methanogenic incubations but stimulated toluene biodegradation under sulfate-reducing conditions. Again, the inhibitory effect was attributed to the preferential utilization of ethanol.

Because of the absence of field evidence and limited laboratory testing, general conclusions can not be made regarding the effect of ethanol on BTEX biodegradation in subsurface environments contaminated with ethanol-blended fuels. The laboratory-based

information obtained thus far suggests that ethanol may in some instances prevent BTEX biodegradation. However, the idea that ethanol prevents BTEX biodegradation depends on the length and duration of the ethanol plume emanating from a gasoline source. Although field studies documenting ethanol biodegradation were not found, the fate of methanol and MTBE in the subsurface has been evaluated (Barker et al., 1990) due to the environmental concerns associated with the release of MTBE and methanol-blended gasoline into subsurface aquifers. In these studies, the effect of MTBE and methanol on the migration of BTEX hydrocarbons through a shallow aerobic aquifer was determined. Three aqueous solutions, one containing only BTEX hydrocarbons, the second BTEX plus methanol at a concentration of 7000 mg/L, and the third BTEX plus 289 mg/L MTBE, were injected into the aquifer and allowed to migrate with the natural groundwater flow. Both MTBE and methanol migrated at the same rate as the conservative tracer indicating that these compounds migrated at the rate of groundwater flow. Neither compound had a noticeable effect on the rate of migration of the BTEX, but methanol decreased the disappearance of benzene and m-xylene (by ~30%) relative to the benzene and m-xylene plumes that did not contain a gasoline oxygenate or contain MTBE. This effect was not observed for MTBE, which was found to be recalcitrant during the field tests. The increased persistence of BTEX in the methanol plume was not specifically evaluated but was speculated to be attributed to the removal of oxygen during methanol biodegradation. Because of the low solubility of oxygen in water (~12 mg/L), Barker (1990) calculated that only a small amount of the injected methanol at a concentration of 7000 mg/L would consume all the available dissolved oxygen in and along the flow path of the aquifer thereby inhibiting aerobic biodegradation of BTEX.

Because methanol and ethanol have similar chemical structures, aqueous solubility, partitioning characteristics, and susceptibility to biodegradation, the impact of methanol on the migration and biodegradation of BTEX in aquifers is likely to be similar to that of ethanol.

The greatest impact would occur if BTEX biodegradation were completely inhibited in the presence of ethanol either due to preferential biodegradation or the exhaustion of available electron acceptors during ethanol metabolism. In either case, the extent to which ethanol would extend the size of a BTEX plume is dependent on how far and for what period of time the ethanol and BTEX plumes are in contact.

Poulsen *et al.* (1992), found that it is unlikely that ethanol will persist in gasoline-contaminated groundwaters for a significant time relative to BTEX. They suggest this is because of the relatively short time required for ethanol to completely leach from pools of nonaqueous gasoline relative to hydrocarbons and the rapid rates of ethanol biodegradation. During the field injection experiments discussed above (Barker *et al.* 1990), methanol was completely removed from the groundwater within 470 days. Similar rates of methanol biodegradation have been observed in subsurface sediments at concentrations up to 1000 mg/L at rates sufficient to remove this concentration in less than one year (Novak *et al.*, 1985). Since the biodegradability and transport behavior of ethanol are similar to methanol (suggesting similar residence times in subsurface aquifers), it is anticipated that the effects of ethanol due to direct contact with BTEX will be short-lived.

Remediation

Ethanol.

Although it is unlikely that ethanol will migrate or persist in gasoline impacted aquifers to the extent of BTEX hydrocarbons, there may be impetus to treat source areas where ethanol is most likely to occur, especially if the spill threatens groundwater drinking wells or environmentally sensitive surface waters. In such instances, the focus will likely be the removal of gasoline hydrocarbons using traditional groundwater treatment technologies including air stripping and activated carbon. While air stripping is relatively effective at removing volatile hydrocarbons, this process is inefficient at removing ethanol from water due to its low partitioning from the aqueous to the gaseous phase. Treatment with activated carbon will also be ineffective due to the high water solubility and low sorption coefficient of ethanol. Thus, traditional technologies for the treatment of gasoline contaminated water will not be useful for ethanol.

Fortunately, biological treatment systems including bioreactors and biologically activated filters are likely to be effective for the treatment of ethanol contaminated groundwater. Ethanol has been shown to be effectively removed from synthetic brewery waste water (Wu and Hickey, 1996) and other ethanol-containing waters (Lettinga *et al*. 1981) using methanogenic up-flow anaerobic sludge blanket reactors. Denitrifying and aerobic microbial treatment systems are also likely to be efficient in treating ethanol-contaminated water due to the rapid rates of ethanol biodegradation under these electron accepting conditions (Hallin and Pell, 1997; McKinney and Jeris, 1954).

MTBE.

MTBE in soil, as the result of a gasoline release, may separate from the rest of the gasoline, reaching the groundwater first and dissolving rapidly. Once in the groundwater, MTBE travels at about the same rate as the groundwater whereas benzene and other gasoline constituents tend to biodegrade and adsorb to soil particles.

MTBE-contaminated soil does not pose a significant environmental threat because the MTBE can be removed from the soil by soil vapor extraction (SVE) or low-temperature thermal desorption (LTTD) (USEPA, 1998b:2). However, once MTBE reaches the groundwater it can be problematic. MTBE's high water solubility, low rate of soil adsorption, and low rate of biodegradation can make treating groundwater contaminated with MTBE more expensive than treating groundwater contaminated with gasoline that does not contain MTBE.

Soil Column Testing

Experiments can determine the release, transport, retardation, and transformation parameters of solutes under flow conditions. Knowledge of these processes plays a crucial role in the estimation of the risk of soil and groundwater pollution. These experiments can be conducted in a controlled environment, or in a field environment. The focus of this research was based upon experiments using a soil column system in a laboratory setting. Specifically, this research consisted of feeding an ethanol/water mixture through a soil column system to determine if degradation occurred and if the system performed correctly. The degradation was due to microorganisms in the soil.

This section gives background of why soil columns were used, their components, and how they have been used.

Background.

It is very difficult to conduct *in-situ* (in the natural or original position) experiments involving microorganisms. The addition of microorganisms, or transporting a sample back to the lab for analysis, may disrupt the complex environmental system. Because of these problems, most transport studies are conducted using either columns or lysimeters. Only columns will be discussed here. These systems are contained, may be designed to facilitate sampling at a variety of depths, and can be manipulated more readily to determine the influence of specific factors on transport (Maier *et al.*, 2000:166).

Columns.

The columns used in contaminant transport studies are usually made of plastic, glass, metal, or a combination of these materials. Screens or filters placed at the end of the column retain the porous medium packed into the column and allow regulation of flow conditions. Screens can be made of plastic, stainless steel, or nylon mesh. The column is packed with the porous medium of interest, which is typically glass or silica beads, natural soil, or vadose zone materials. The column must be packed carefully in order to minimize formation of macropores that result in preferential flow, and to obtain a specific bulk density. Sandy soils are often used for contaminant transport studies to allow reasonably high flow rates and to prevent column plugging (Maier *et al.*, 2000:166). Soil from the site of interest should be used as the packing material in order

to obtain the most relevant information when performing site specific research. In order to mimic the natural soil system, the soil should be packed to the bulk density of that in the natural environment. Another approach is to use intact soil cores. These cores can be obtained by driving an empty column into the soil and carefully extracting it. Soil cores are very difficult to keep intact and susceptible to compaction (Maier *et al.*, 2000:166).

Pumps.

Pumps deliver fluid to the column. For saturated flow systems, the fluid is usually delivered from the bottom to the top of the column to help displace gas bubbles within the column. For unsaturated columns, a vacuum chamber located at the outflow end of the column can be used to provide a near-constant degree of saturation throughout the column. Flow for unsaturated columns is generally from top to bottom because for unsaturated flow, gas is, by definition, present (Maier *et al.*, 2000:166).

Column Inoculation.

Inoculation of a vertical column for a contaminant transport study can be accomplished in a variety of ways. It can be done by inoculating the soil with non-indigenous microorganisms or by promoting the growth of indigenous microorganisms by adding compounds to the soil. The medium can either be saturated or unsaturated. Tipton *et al.* (2003) conducted a saturated soil column experiment to examine the effect of biodegradation on perchlorate fate and transport in soils. Pulses of solution containing perchlorate were applied to saturated soil columns at steady state water flow. Abu-Ashour and Shahalam (2002) inoculated their unsaturated soil column by mixing diesel obtained from a local gas station with the soil before it was packed into the column.

They then applied water to the top of the column. Another method (Adam *et al.*, 2002) was to add ten milliliters of diesel fuel, using a syringe, to the top of the column and to allow penetration for 30 min. Then 50 mL of water was added to wet the column, followed by 2 liters of deionized water by inverting a 2.5 liter plastic bottle into the top of the column. There are many ways to inoculate the column with microorganisms, and once it is inoculated, the column is usually maintained under flow conditions for a specified period of time.

Sampling.

Sampling of the column can be achieved in a variety of ways. Destructive sampling involves disassembling the column to obtain samples. Destructive sampling disrupts the matrix to such an extent that the experiment must then be terminated. Adam *et al.* (2002) used 4 cm (inside diameter) polythene drain pipe cut into ten, 10-cm long sections. The sections were sealed together using waterproof tape. The column was dismantled one section at a time and a 40-g sub-sample was taken from each section. Nondestructive sampling methods include monitoring the effluent from the column or using sampling ports to assess the presence of the microbe, nucleic acid, or contaminant of interest from a site within the column. Ports located at a variety of depths along the column can be used for the introduction of a syringe to sample the soil solution or as sites for the removal of small soil cores. Trevors *et al.* (1990) monitored the percolation water exiting their soil columns for bacterial counts before destructively sampling columns at designated time intervals. In all types of sampling, whenever possible, analysis of samples should be limited to portions of the sample not in immediate contact with the

column surface, because transport at this interface may be different from that through the matrix itself.

Justification

The fate and transport of ethanol in the environment is well understood; however, the interactions between ethanol and other gasoline constituents and their resulting fate and transport are not well understood. Corseuil et al. (1998) conducted a laboratory study and found that ethanol retarded BTEX aerobic biodegradation and rapidly reduced oxygen concentrations. Hunt et al. (1997) found that the degradation of toluene was completely inhibited until all the ethanol was degraded.

Contrary to early reports of MTBE's inability to degrade, recent research suggests that MTBE is indeed degradable by a wide range of different microorganisms. However, more research is required to better understand the factors limiting MTBE biodegradation in the environment (Deeb *et al.*, 2000:183).

In the case of oxygenate mixtures, several researchers have investigated the kinetics of ETBE, MTBE and TAME biodegradation in mono-substrate systems, both in aerobic and anaerobic conditions (Hardison *et al.*, 1997; Kharoune *et al.*, 1998; Mormile *et al.*, 1994; Steffan *et al.*, 1997; Suflita and Mormile, 1993) and one study encompassed all three oxygenates (Kharoune *et al.*, 2001). Information on the relative biodegradation rates of a mixture of oxygenates (ethanol, MTBE, BTEX, etc.) is important as it is likely that all of these compounds will be encountered at contaminated sites.

Further research is needed to better understand the fuel oxygenates and how they react under different conditions. The impact of ethanol on BTEX and/or MTBE needs

further research to verify the laboratory study conducted by Corseuil et al. (1998). Other ethanol studies that need further research are the occurrence of ethanol in water in states using gasoline containing ethanol and the toxicity of ethanol in the source area. Kharoune *et al*. (2001) suggests that more complete studies about the influence of oxygen concentration on the oxygenate degradation would be useful. The Blue Ribbon Panel on Oxygenates in Gasoline (USEPA, 1999:9) recommends accelerated study of the health effects and groundwater characteristics of ethers (e.g. ETBE, TAME, and DIPE) before they are allowed to be placed in widespread use.

III. Research Methodology

Introduction

This chapter describes the study methodology – the soil column system, the sampling method, and the method of analyzing samples taken from the soil columns.

A series of soil columns were constructed to conduct laboratory experiments on fuel oxygenate (ethanol) degradation under differing soil oxygen concentrations. An oxygen sensor system was used to analyze the oxygen content in the columns and to control the feed mixture into the columns.

Tracer tests, using sodium chloride (NaCl), were run to obtain information on column hydraulics and to ascertain how similarly the columns behaved hydraulically. These tracer experiments were conducted using a YSI conductivity probe to measure salt concentrations. A gas chromatograph (GC) was used to analyze influent and effluent samples to determine the amount of oxygenate in the sample. A combination of ethanol and water was continuously pumped into the tops of the columns to simulate exposure to the soil microorganisms in a soil system.

Experimental Setup

Column Setup.

The soil column experimental setup consisted of eight, 8-inch diameter PVC columns that were eight feet tall and capped at the bottom. Each column was constructed in the same manner. Three holes were drilled in each column, two as ports for silicon tubing to be inserted into the columns, from which oxygen sensors could measure the soil

oxygen content, and an effluent port. The bottom hole was 8.5 inches from the bottom, the middle hole was 2 ft from the bottom, and the top hole was 6.5 ft from the bottom of the column. Each hole was 1-5/8 inches in diameter and plugged with a rubber stopper. In the bottom hole a 6 inch long, ½ inch diameter steel pipe nipple was put through the rubber stopper to form the effluent drain. In the middle hole, two 6 inch long, ¼ inch diameter pipe nipples were put through the rubber stopper and connected to a 3 ft coil of reinforced silicon rubber tubing on the inside of the column. In the top hole, four 6 inch long, ¼ inch diameter pipe nipples were put through the rubber stopper. Two of the pipe nipples were connected to a 3 ft coil of reinforced silicon rubber tubing and the other two were connected to a 4 ft coil of non-reinforced silicon tubing. Silicon tubing was used because it is permeable to gasses. Only the top port was used in this experiment; the bottom two ports are functional but need oxygen sensors. Figure 4 shows the schematic of the feed system which includes the top port assembly.

Coarse (~1" diameter) drain rock was poured into the columns to a depth of one foot. Then 5.5 feet of sandy soil was poured into the columns in 1 foot lifts. The columns were saturated with tap water through the effluent drain forcing the water up from the bottom.

Feed System

Purpose.

The feed system was a combination of components that worked together to add the correct amount of chemicals into the columns to keep the oxygen content in the soil at a specified, constant level. When the oxygen content in the soil rose above the set point,

the computer turned the feed pumps on and the chemical/water mixture was added to the top of the column.

Theory.

The feed system (Figure 4) used a personal computer to control the experiments and record data. GAST DDL series air pumps forced air through the silicon tubing loops inside the column, 12 inches below the top of the soil, and back out to Dririte desiccant driers before connecting to Japan Battery Co. Ltd oxygen sensors. The oxygen sensor read the oxygen content in the loop and sent the reading back to the computer every 15 seconds. The computer compared the reading to a user defined set point. If the oxygen concentration was greater than the set point, the computer would turn a set of pumps on that would add a chemical mixture to the top of the column. The Masterflex C/L variable speed pump fed neat ethanol that was stored in 40 mL vials. The Masterflex L/S fixed flow pump fed the tap water that was stored in 5 gallon buckets. A mixing tube combined the chemicals and the water, which fed into the top of the columns.

This process ran continuously for approximately eight weeks, but the oxygen concentrations never dropped low enough to meet the user defined set points. The system was taken off-line for approximately two weeks to troubleshoot the problems. Air had entered the system and interrupted the flow. Also, the pumps were set at such a low speed that they were stopping on their own. The pump tubing was replaced with continuous lines to ensure air could not enter the system. The pump speeds were adjusted so they would not stop on their own. The system was then turned back on for four weeks and it functioned properly, with the oxygen concentrations dropping and converging to the user defined set points.

Data Collection.

The computer that controlled the feed system took readings from the oxygen

sensor every 15 seconds. Therefore, if pumps for a particular column were activated,

they would run for at least 15 seconds or in 15 second intervals until the oxygen content

dropped below the set point. The computer recorded the oxygen sensor reading every 90

seconds into a file. Date, time, and oxygen reading for each column were recorded. A

sample of the graphical output from the oxygen sensor data is shown in Appendix W.
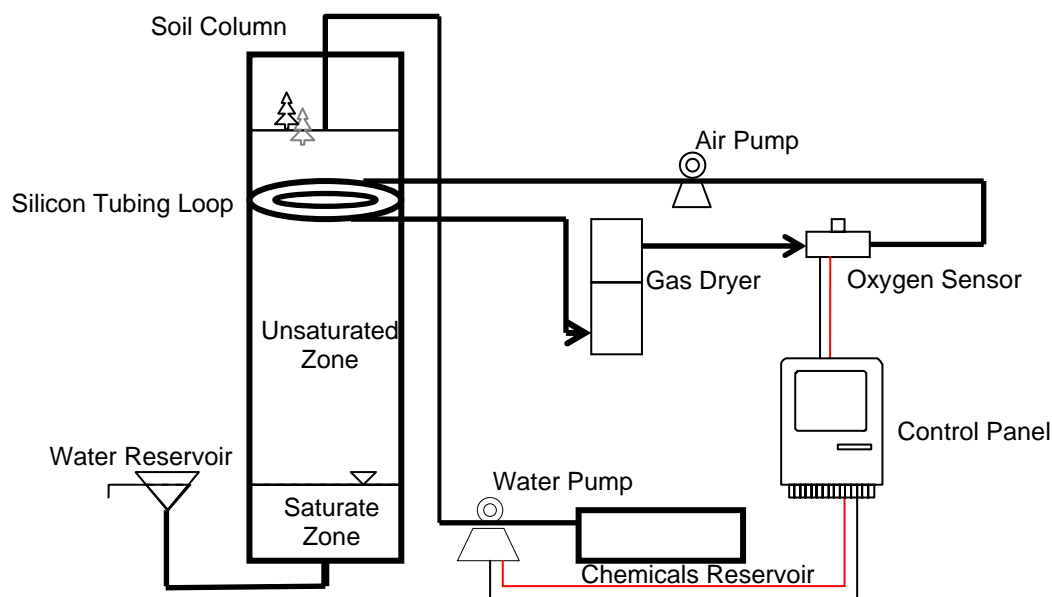
Figure 4. Schematic of the In-situ Oxygen Sensor for Use in Bio-treatment of Fuel
Oxygenates in Groundwater.

Pump Flow Rates

Two types of pumps were used in this experiment, Masterflex L/S fixed flow pumps and Masterflex C/L variable speed pumps. The pump flow rates were necessary to calculate the concentration of ethanol fed into the top of each column. The calculated concentration was used as a check to compare with the actual GC results to verify the equipment was working properly. Sample calculations of the concentration of ethanol to be fed into the top of each column are found in Appendix B.

The fixed flow pump used 1/8 inch inside diameter (ID) Norprene tubing. The average flow rate for the eight pumps (one per column) was approximately 28.2 mL/min. This was checked both before and after the tracer tests were performed. The pump flow rates were checked for each pump by starting with one liter of water in a graduated cylinder and pumping for 30 minutes. The pump flow rate was calculated by dividing the volume pumped by 30 minutes. The actual flow rates for each pump can be found in Appendix A.

The variable speed pumps used 0.89 I.D. Viton pump tubing. The flow rates for the ethanol varied initially, as the correct setting on the variable speed pump was adjusted. Once the adjustment was set, the average flow rate for the eight pumps (one per column) was approximately 0.032 mL/min. The actual flow rates for each pump can be found in Appendix A.

Tracer Tests

Tracer tests were run on each of the columns to determine hydraulic retention times and pore volumes for the columns. The retention time and pore volume for each column was calculated and is tabulated in Appendices M and S. It was necessary to

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know the column hydraulics because the fate of the contaminants depends on both the chemical nature of the contaminant and how the chemical flows through the columns. It was also important to know how these characteristics compared between the columns. Ideally, the columns were to be constructed exactly alike. This would help ensure replicate data. However, as discussed in Chapter 4, identical columns are an idealization.

The tracer tests used 22.4 grams of NaCl in each 5 gallon tap water reservoir bucket for each of the columns. Each bucket was filled with 18 liters of tap water, for an average initial concentration of 1365 mg/L NaCl (the actual initial concentrations can be found in Appendices D through L). The NaCl solution was continuously pumped into the tops of the columns at an average flow rate of approximately 28.2 mL/min (the actual flow rates can be found in Appendices D through L). Once the NaCl solution had been fed into the columns, tap water was pumped into the columns at the same flow rates. Effluent samples were taken approximately every hour for 24 hours and then every 12 hours for the next 48 hours. These data are tabulated in Appendices D through L.

The effluent was analyzed using a YSI conductivity probe, an indirect measure of the amount of salts in the solution. Figure 5 shows a calibration curve used to convert conductivities into concentrations. Relative concentration (actual concentration divided by initial concentration) versus time was plotted and the Method of Moments was used to determine the retention times and pore volumes. This method is similar to the impulse-tracer method found in Clark (1996). The zeroth and first moments were found using the breakthrough curve data. The normalized first moment (First moment/zeroth moment) was used to calculate the retention time. The pore volumes were calculated by multiplying the retention time by the flow rate (assuming the flow rate was constant).

The mass balance error was also calculated, to see how much of the sodium chloride mass was recovered. This was calculated by subtracting the output mass from the input mass and dividing that by the input mass. The output mass was the zeroth moment multiplied by the flow rate and the input mass was the initial concentration multiplied by injection time multiplied by flow rate. A negative mass balance error signifies that the output mass is greater than the input mass. These calculations were based on the assumption that the flow rates were constant throughout. These results are tabulated and represented graphically in Appendices D through M.

Calibration curves were created before and after the tracer test experiment to see if the YSI conductivity probe's readings changed during the experiment. Standard NaCl solutions were prepared using certified A.C.S. crystals from Fisher Scientific. All standards were made in 100 mL glass bottles with de-ionized water and capped with glass stoppers. The standards were made by adding 50 mg, 100 mg, 150 mg, and 200 mg NaCl into 100 mL de-ionized water to create the following concentrations of NaCl: 500 mg/l, 1000 mg/l, 1500 mg/l, and 2000 mg/l. Figure 6 shows a calibration curve that gives the conversion from conductivity to concentration of NaCl as: concentration (mg/L NaCl) = 0.48 * conductivity (μS/cm). The calibration was checked before and after the tracer tests were performed (Appendix C).

The calibration curves mentioned above were developed using de-ionized water, however, the actual tracer test experiments were conducted using tap water. The tap water contains natural salts that add to the conductivity. Therefore, a calibration curve using tap water was calculated. This eliminated having to subtract off a baseline for the salts in the tap water that was flowing through the columns. Figure 6 shows a calibration

curve that gives the conversion from conductivity to concentration of NaCl as:

concentration (mg/L NaCl) = 0.54 * conductivity (µS/cm) – 438.35 mg/L.
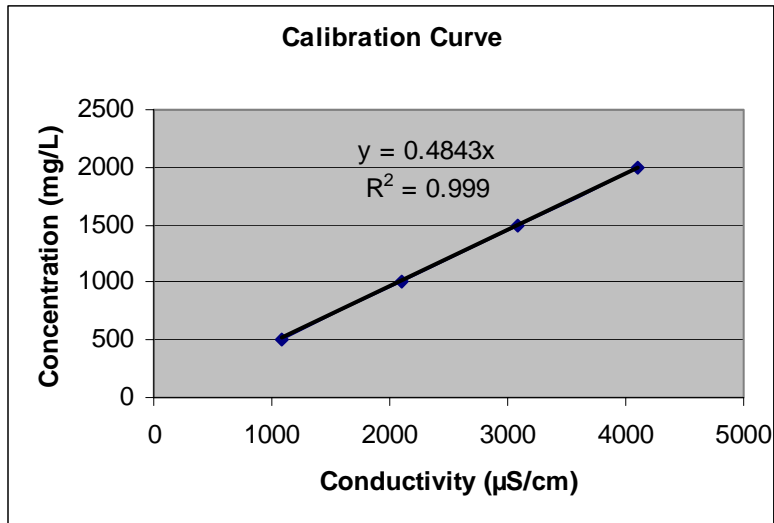
Figure 5. Calibration Curve for YSI Conductivity Probe (w/ de-ionized water)
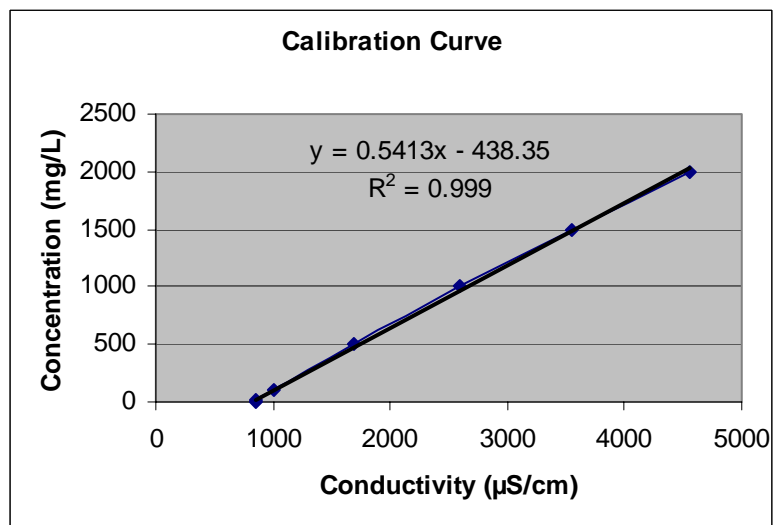
Figure 6. Calibration Curve for YSI Conductivity Probe (w/ tap water)

The 438.35 mg/L accounts for the presence of natural salts in the tap water and simplifies the conversion to concentration of NaCl. The calibration curves and supporting data can be found in Appendix C.

Sampling Method

The influent and effluent for each of the eight columns were sampled once a day for approximately two months. These samples were analyzed with the GC to see if there was a difference in the ethanol concentration in the influent and the ethanol concentration of the effluent. Duplicate samples were taken the first week. Data were consistent, so only one sample per column per day was taken after that. Using the data from the tracer tests, I was able to calculate the retention times for each of the columns (Appendix M). Sampling once a day ensured that at least one pore volume had time to flow through the column before the next measurement was taken. This was important because if two samples were taken in the same pore volume, they may be too closely related and the data would be biased. It was important to sample the influent in order to know exactly what was going into the top of the column. Since the flow rates for both sets of pumps were known, the concentration of ethanol going into the tops of the columns could be calculated. If the measured influent concentration and the calculated influent concentration were not close, this meant there was a problem with the equipment.

The effluent port consisted of a 6 inch long, ½ inch diameter steel pipe nipple placed through a rubber stopper. A 12 inch piece of ½ inch diameter vinyl tubing was connected to the pipe nipple. A ½ inch "T" was placed at the end of the vinyl tubing and another piece of ½ inch vinyl tubing was placed on a second arm of the "T", which

drained into a waste bucket. The remaining arm of the "T" was used as the sampling port. Figure 7 below shows the effluent port assembly.

A ten milliliter syringe was used to sample the effluent. Following two flushes of the syringe with de-ionized water, it was inserted into the open "T" arm of the effluent port at the bottom of each column. The first 5 mL volume extracted from the effluent port was discarded. This prevented any residual deionized water from diluting the sample. After that, 5 mL was extracted from the effluent port, 2 mL of which was used to fill a 2 mL glass vial. All vials were filled to the brim, creating a fluid meniscus at the top to prevent any air bubbles from entering the vial when capped.

The sampling procedure for the influent was a little different. Since the influent solution was being pumped through a mixer, there was no reservoir to extract from. The tubing that the chemical mixture was pumped through into the top of the column was placed in a 2 mL glass vial and the vial was filled using the same technique as was used with the effluent samples to prevent air from entering the vial.
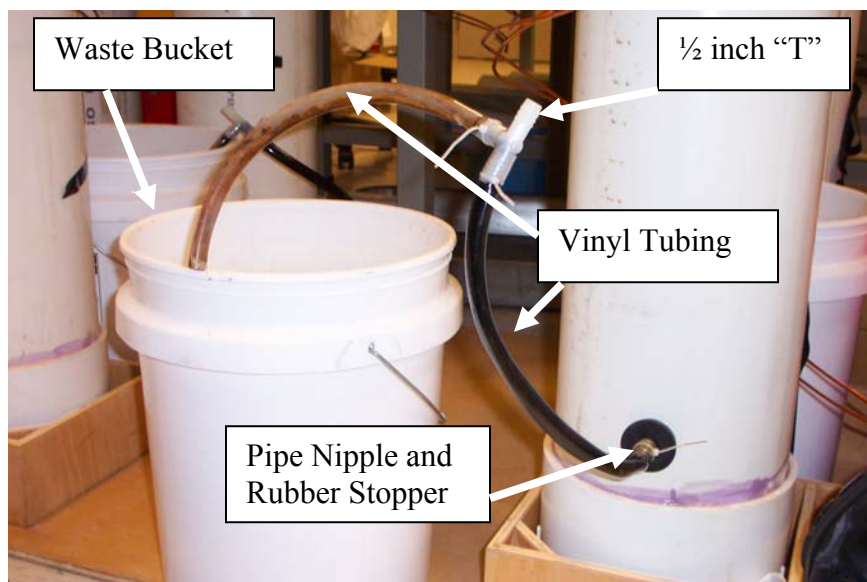
Figure 7. Effluent Port Assembly

Preparation of Standards

Standard solutions for ethanol were prepared from reagent, HPLC grade, denatured ethanol from Sigma-Aldrich of Milwaukee, WI. All standards were made in 100 mL glass bottles with de-ionized water and capped with glass stoppers. Gas-tight syringes (10 mL and 10 uL) were used to transfer the ethanol solutions and de-ionized water into the vials.

Standard solutions were prepared by serial dilutions. A 1000 mg/l ethanol solution was used to make the other standards. A 10 µL gastight syringe was used for the transfer of fluids. The syringes were rinsed 2 times with de-ionized water and dried between each use. Calibration curves were then created after running each of these standard concentrations through the GC. The curves were forced through zero resulting in improved R-squared values for each of the analytes of over 0.999. These calibration curves can be found in Appendix T.

A series of standards was also made using the effluent of columns 1 and 2 when tap water only was flowing through them. The same procedure as above was used to make the ethanol solutions. This calibration curve can also be found in Appendix T.

Gas Chromatograph (GC) Method

An HP 5890 Series II Gas Chromatograph (GC) was used to analyze the components of each sample. Five meters of the Connex 160-2325 (Deact Fused Silica, length: 5 m, ID: 0.32 mm) guard column was connected to a DB-624 (123-1334), JW Scientific (Length: 30 m, ID: 0.32 mm, Film: 1.8 um) column which was then connected to the Flame Ionization Detector (FID). The FID was used because of its ability to detect the fuel oxygenates. The GC analytical operating parameters were originally selected

based on the method found in Wang (1999). However, based on trial and error, the method was adjusted to obtain better peak clarity and separation. The modified operating parameters are listed below in Table 4.

The ChemStation software package version 4.1 was used on a desktop computer to run the analytical sequence for the AutoSampler, and the GC. The software plotted the chromatogram and integrated the chromatogram peaks. Excel was used to calculate the concentration of ethanol using the area under the curve based on the standard calibration curves.

Table 4. Autosampler and GC Conditions

Gas Chromatograph	HP 5890 Series II
Autosampler	HP 7673A Automatic Injector
	Syringe: gas tight syringe with teflon tip (5uL)
	Injector (2) parameters:
	Mode: 0 (0=normal, 1=on column)
	Pre-injection sample wash: 3
	Viscosity: 5
	Sample pumps: 6
	Sample volume: 0.5 uL
	Post-injection acetone wash (A): 3
	Post-injection MilliQ H_2O wash (B): 6
	Injections per bottle: 1
Data System	Waters, Millennium
Flame Ionization Detector	
Temperature	250 C
Carrier Gas (H_2)	35 ml/min @ 40 C, 15 psi
Split Vent	20 ml/min @ 40 C
Make-up Gas (N_2) w/H_2	23 ml/min @ 40 C
Carrier Gas (H_2) + FID H_2	40 ml/min @ 40 C, 40 psi
Septum Purge	3 to 5 ml/min @ 40 C
Air	350 ml/min @ 40 C, 36 psi
Injector Temperature	175 C
Injection Volume	1 uL
Splitless Injection	Purge valve (6) on at 0.5 min
Injection Liner	Restek 2mm ID Splitless Sleeve for HP GCs, Prepacked with FS wool, Cat.# 20713-200.5

Column	DB-624 (123-1334), JW Scientific
	Length: 30 m, ID: 0.32 mm, Film: 1.8 um
Guard Column	Connex 160-2325 (Deact Fused Silica)
	Length: 5 m, ID: 0.32 mm

GC Conditions
 Programmed Oven

Oven Initial Temperature	40 C
Initial Time	1 min
Program Rate	10 C/min
Final Temperature	160 C
Final Time	0 min
Oven Temp Equilibrium Time	1 min

Integrator

Threshold	4
Attenuation	2
Peak Width	0.04
Chart Speed	0.3 cm/min

The integrator was used only as a charting device, to provide ready access for viewing

instrument output, not quantitation.

The method detection limit was calculated by taking replicates of a known

concentration (1 mg/L), and then calculating with the following equation:

$$MDL = SD \times t_{0.99}$$

where:
$SD = \{\Sigma^{n}_{i=1}(x_i - X)^2 / (n-1)\}^{1/2}$
MDL = method detection limit (mg/L)
SD = standard deviation
$t_{0.99}$ = t-distribution table value for 99% with the degree of freedom (n-1)
x_i = spiking replicates concentration (mg/L) (i = 1 . . . n)
X = the mean of spiking concentrations (mg/L)

The 99% confidence, based on the t-distribution, assumed that the distribution of the low

level spiking concentrations follows the t-distribution. The MDL for the method used in

this experiment was 0.93 mg/L. Appendix AA shows the calculations for the MDL.

IV. Data Analysis

Introduction

This research involved equipment setup/construction and an initial investigation of how the soil column system worked. The column system ran continuously (24/7) for four weeks except for maintenance, refilling the water and chemical reservoirs, and any improvements that needed to be made on the equipment. The system was then shut down for two weeks for modifications and improvements. It was then restarted and ran continuously for four more weeks.

The data collected were from two different types of experiments. Tracer tests yielded breakthrough curves to determine hydraulic characteristics of the columns; specifically retention times and pore volumes. The hydraulic characteristics were also used to compare how similar the columns were to each other. In the second set of experiments, ethanol, mixed with tap water, was fed into the tops of the soil columns to determine how well the soil microorganisms would degrade it. The soil oxygen content was measured to show if there was microbial activity. A drop in soil oxygen content was an indication that microbial activity was occurring. The influent and effluent samples were analyzed using a gas chromatograph to determine how much degradation was occurring.

Tracer Test Results

A breakthrough curve was calculated as the NaCl solution passed through each column. Figure 8 below shows the breakthrough curve for Column 2, resulting in a calculated retention time of 9.3 hours and a pore volume of 15.7 liters. The mass balance

error was only 0.57% for column 2. The retention times, pore volumes, and mass balance errors for each of the columns can be found in Appendices M and S.
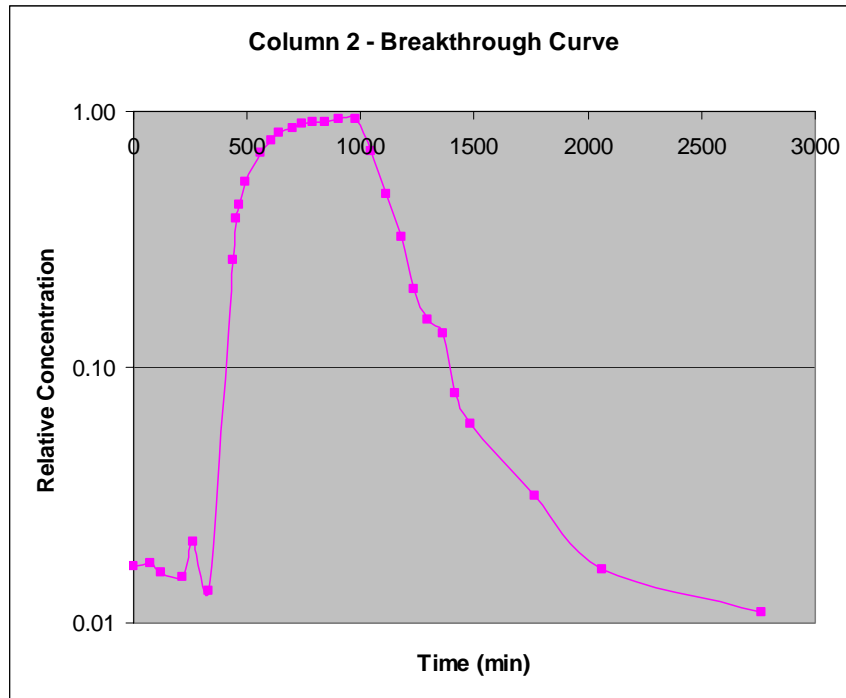
Figure 8. NaCl Breakthrough Curve – Column 2

Columns 1 through 4 produced similar breakthrough curves with low mass balance error (<7%). The breakthrough curves for those columns look like Figure 8 above, the graphs are smooth with a slight tailing to the right. However, columns 5 through 8 produced much different results.

The breakthrough curves for columns 5 through 8 started out smooth, but as the concentrations began to decrease, there was an extended tailing to the right. Figure 9 shows the breakthrough curve for Column 5. The tailing affect seen in columns 5 through 8 is not unheard of. van Genuchten and Wierenga (1976) suggested that experimental breakthrough responses exhibit highly asymmetric or nonsigmoid profiles, commonly termed tailing, and that tailing may be attributable to the slow diffusion of

solute into zones of immobile water. They also hypothesized that these zones result from

soil aggregation, slow flow, or unsaturated flow (van Genuchten and Wierenga, 1977).

From the breakthrough curve for Column 5, a retention time of 16.9 hours and a

pore volume of 27.3 liters were calculated. The mass balance error was 40.8% for

column 5. The extended tailing causes a large increase in the retention time and pore

volume. The extended tailing made it appear as though there was still NaCl mass present

in the system. However, this is not what I found when I calculated the input and output

masses. As the mass balance errors show (Appendix M), most are negative. The

negative mass balance error suggests that the output mass is greater than the input mass,

which is opposite of what the extended tailing suggests. The only explanation that I have

for this is that the flow rates were not constant over time, as assumed.
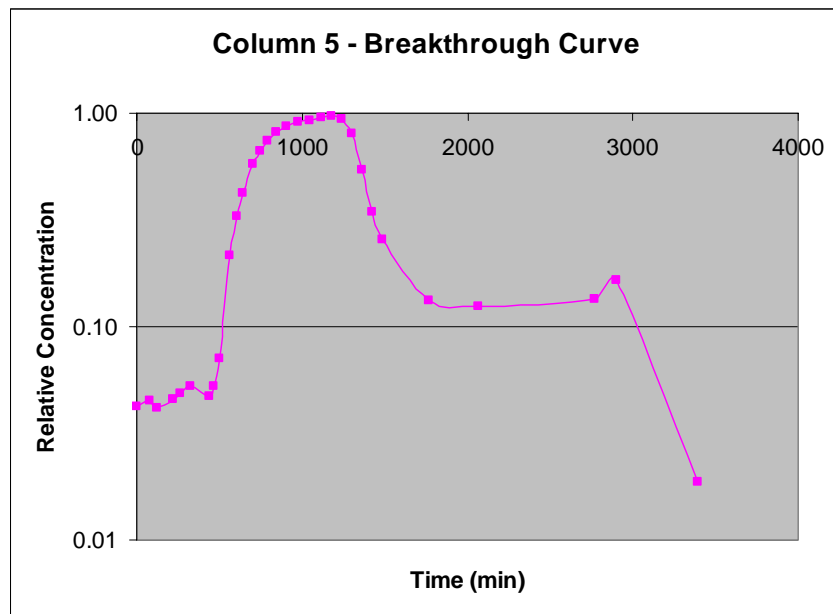
Figure 9. Breakthrough Curve – Column 5

After analyzing the data from Column 5 and using Columns 1 through 4 as

references, I adjusted the baseline and interpolated the end points on the right tail of the

graph. The base-line was adjusted because, even after accounting for the salts in the tap water, the effluent NaCl concentrations did not start out at zero. They were actually much higher than the concentrations at the end of the tests. The baseline was adjusted to bring the initial effluent NaCl concentrations down to approximately zero. The last few points of the curves were interpolated to get rid of the tailing and make them look similar to the curves for columns 1 through 4. A line segment was found and extended down along the slope at which the line began decreasing.

Figure 10 shows the interpolated and base line adjusted breakthrough curve for Column 5. This breakthrough curve now looks very similar to those of Columns 1 through 4. The retention time was 13.0 hours, the pore volume 21.0 liters, and the mass balance error was only 5.95%. This was a significant improvement and more closely resembled what was expected. The tabulated results for the original tracer test data can be found in Appendices D through L, with the retention times, pore volumes, and mass balance error in Appendix M. The tabulated results for the modified tracer test data can be found in Appendices N through R, with the modified retention times, pore volumes, and mass balance error in Appendix S.
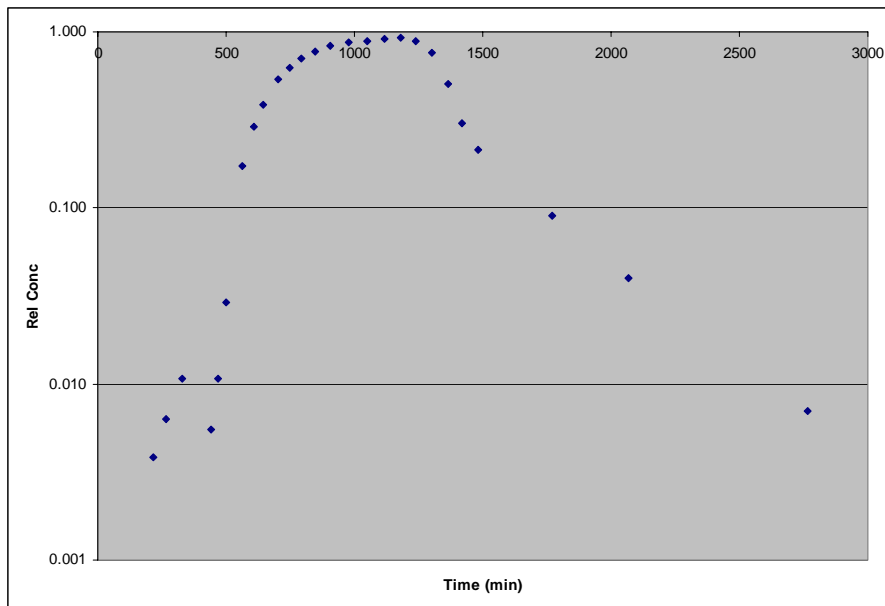
Figure 10. Interpolated and Base Line Adjusted Breakthrough Curve – Column 5

GC Results

The influent and effluent for each of the columns was sampled and analyzed for

ethanol using a gas chromatograph (GC). The results of the influent GC analysis are

shown in Figure 11. Examples of the influent and effluent output chromatograms can be

found in Appendices Y and Z.
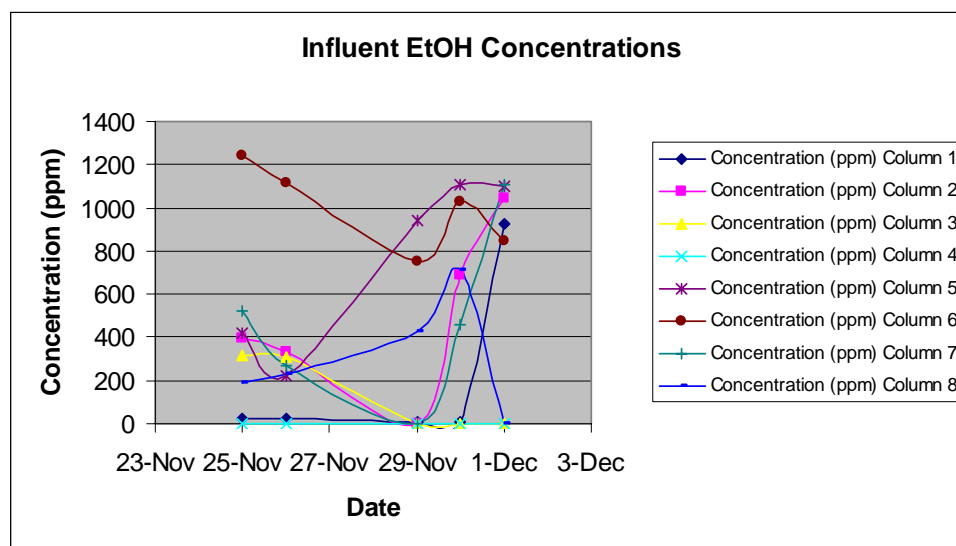
Figure 11. Influent Ethanol Concentrations

Figure 11 shows that the influent ethanol concentrations were variable, exposing a problem in the experimental setup. The calculated influent concentrations (Table 5) show the expected influent concentrations. The lines in Figure 11 should have been straight for the concentrations shown in Table 5

Table 5. Calculated Influent Ethanol Concentrations

Column	Concentration (mg/L)
1	919.7
2	865.1
3	725.7
4	389.5
5	932.7
6	462.5
7	1116.9
8	1028.8

Effluent results (Figure 12) show low concentrations of ethanol initially, then an increase. Some time is required for the population of microorganisms to develop and become acclimated to the new organic chemical in their environment. A decrease in ethanol concentration is expected as the microorganisms adapt to the new environment. Since ethanol is a small, soluble straight chain organic, it is very easily broken down. However, Figure 12 shows this did not occur. Initial effluent ethanol concentrations started low, and then some, but not all of the effluent sample concentrations, increased. Also, none of the effluent concentrations seemed to go back down. Influent concentrations were not constant, which may have caused some of the problem. Another reason effluent concentrations did not go back down, may be because the microbial

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population had not had enough time to establish itself and adapt to the new chemical in their environment.

Because the influent and effluent concentration versus time profiles did not behave as anticipated, the system was shut down for two weeks for troubleshooting. The experimental setup was closely examined and modifications were made.
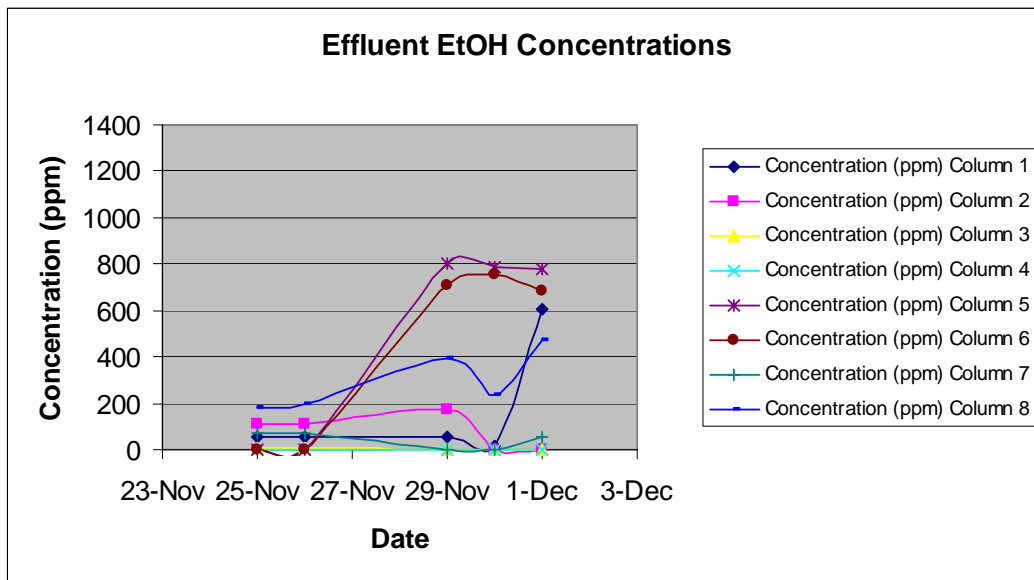
Figure 12. Effluent Ethanol Concentrations

The tubing that ran from the ethanol to the mixing tube contained many breaks and "T's". They were replaced with continuous tubing lines for each ethanol vial. The tops of the vials that contained the ethanol were not sealing around the tubing that entered the vial. The tops were replaced with a Teflon lined septum that sealed around the tubing that entered the vial, reducing the chance for air to enter the system. These modifications didn't seem to solve the problems with the influent concentrations. As Figure 13 shows, the influent samples were still variable; however they showed a more consistent trend than the data in Figure 11. However, the modifications made a significant difference in the effluent concentration data. After the modifications, the effluent concentrations were

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below the method detection limits except for column 6, which had experienced a spike in

feed (Figure 14).
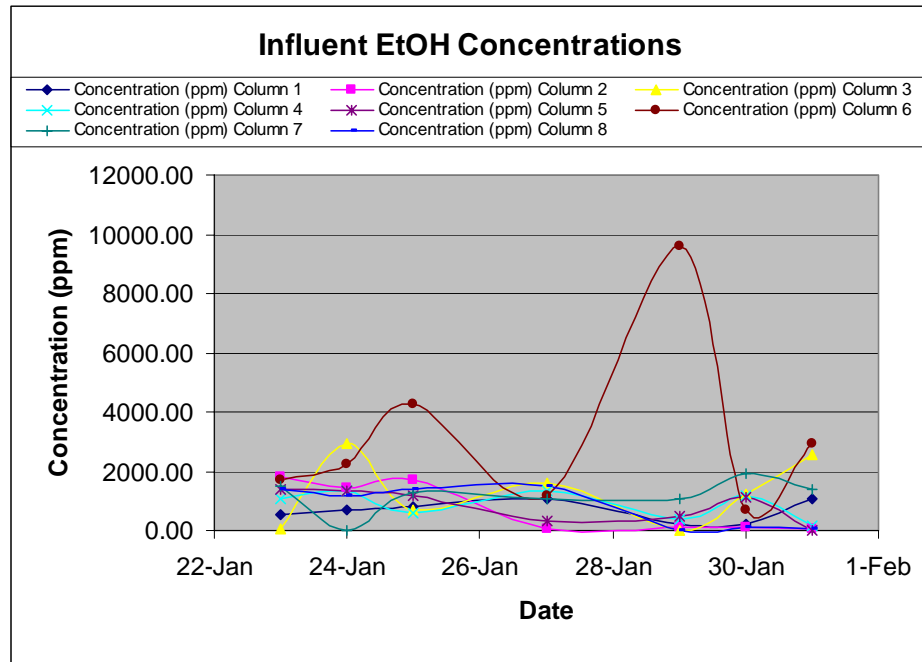
Figure 13. Influent Ethanol Concentrations after Modifications
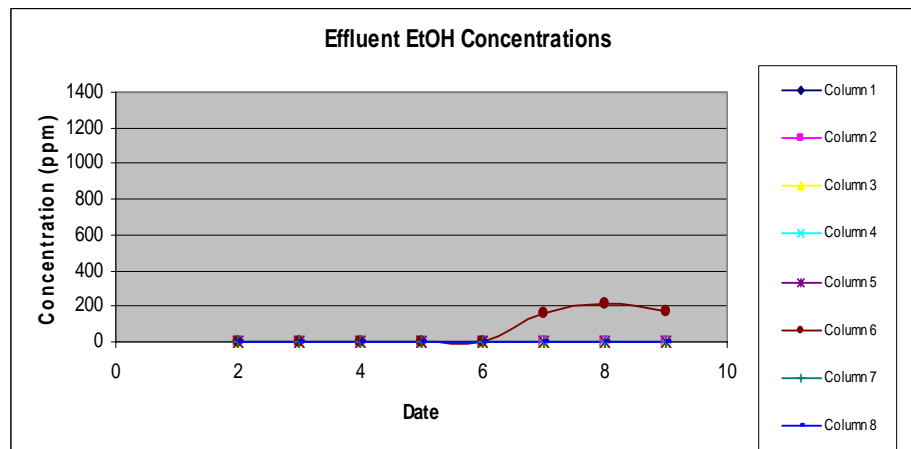
Figure 14. Effluent Ethanol Concentrations after Modifications

V. Conclusions and Recommendations

Introduction

This thesis was to determine how a fuel oxygenate, ethanol, degraded by natural attenuation in laboratory soil columns. In this initial investigation, we constructed the apparatus, got it running correctly, and obtained preliminary data. The research consisted of, 1) a literature review of the current state of alternative fuel oxygenate research, 2) construction of the soil columns, 3) tracer tests to understand the hydraulics of the soil columns, 4) GC analysis of both influent and effluents to determine if degradation of ethanol had occurred, and 5) oxygen content measurements in the soil columns to verify that microbial activity was taking place.

This effort's data gave insight into how the contaminants flow through the soil columns, as well as their retention times and the pore volumes of the columns. The oxygen content data confirmed that microbial activity was taking place. The graph in Appendix V shows a decrease in oxygen content after the ethanol had been fed into the columns, which eventually lead to the convergence of the soil oxygen concentrations to user defined set-points.

Answers to Specific Research Questions

1. *How do the hydraulic properties of the eight columns compare? How alike are they?*

Tracer tests were conducted to determine the hydraulic properties of the eight columns and how alike they were. A major focus in the construction of the eight

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columns was to construct them as similarly as possible. This would ensure that the comparisons between columns were not biased by experimental artifact. The columns were all made with the same materials and the same amounts of aggregate. The pumps and the flow rates were also as similar as possible. Still, it is unlikely that eight hydraulically identical columns could be constructed as there are many uncontrollable variabilities when dealing with soil and microorganisms.

The results from the tracer tests suggest that Columns 1 through 4 had similar hydraulic properties and Columns 5 through 8 had similar hydraulic properties. The data from Columns 5 through 8 were adjusted and interpolated. These modified data closely resembled the data from Columns 1 through 4. However, the hydraulic data gained from these new curves depend on the assumptions I made when I conditioned the data.

The retention times and pore volumes for the original data were within 3.1 hours and 4.7 liters of each other. The retention times and pore volumes for the modified data were within 1.4 hours and 2.0 liters of each other. The fact that the retention times and pore volumes are as close as they are for the columns makes this experimental setup a very useful tool.

Mass balance was achieved for Columns 1 through 4, but not for Columns 5 through 8. The mass balance error achieved for Columns 1 through 4 was less than 7%. Considering that an NaCl solution was used as the tracer and conductivity was used to measure the effluent, 7% appears to be within experimental error. Conductivity is a good approximation for measuring NaCl in a solution; however many potential interferences could bias results at low conductivity. Low conductivity readings could be influenced by many things in addition to the added NaCl.

Based on the low mass balance error and the consistency between the results from columns 1 through 4, it appears that he retention time and pore volume measurements for Columns 1 through 4 are reasonable. However, the retention time and pore volume measurements for Columns 5 through 8 were problematic. The mass balances were poor and the retention times and pore volumes were not consistent with those of columns 1 through 4. The extended tailing on the right end of the breakthrough curve distorted retention times and pore volumes. When the breakthrough curves were modified (baseline adjustment and interpolation of the last few data points), the retention times and pore volumes, as well as the mass balance error, were decreased and were more consistent with the values found in Columns 1 through 4. The mass balance error for the original data was within 17.4%. The mass balance error for the modified data was within 3.8%. Since the data had been modified, these values depend on the assumptions I made when I conditioned the data. However, this does suggest that the experimental setup can be a very useful tool.

2. *Is there evidence that biological activity is taking place in the soil columns?*

Yes, the soil oxygen content readings suggest that biological activity took place. The decreasing trends in the soil oxygen content readings were the strongest proof of microbial activity.

Columns 4 and 6 did not have an oxygen sensor connected to the feed system. All the other columns showed a decrease with time in soil oxygen concentration. Eventually, oxygen columns for all columns converged to the user defined set points

(Columns 1, 2, and 3 at 16% and Columns 5, 7, and 8 at 8%). Appendix V shows the soil

oxygen concentrations for the eight columns. It shows how long it took for the soil

oxygen concentrations to drop and finally converge.

3. *Is there evidence that degradation of ethanol is taking place?*

Gas chromatograph analysis of influent and effluent samples from each of the

eight soil columns showed degradation was taking place. Effluent concentrations were,

generally, much less than influent concentrations. Influent and effluent concentrations

cannot be compared at the same point in time; however, retention times are known and

the readings were taken at intervals that allowed influent to flow through the system and

be analyzed as effluent.

At first, the data were inconclusive as to whether degradation of ethanol was

taking place. Then modifications were made to the system and it was very apparent that

degradation of ethanol was taking place. GC data indicated that influent concentrations

were in the range of the theoretical concentrations that had been calculated and the

effluent concentrations were below the limit of detection for the GC. This suggests,

along with the data from the oxygen sensors, that the microorganisms in the soil were

degrading the ethanol.

4. *Did oxygen concentration have an influence on the degradation of ethanol?*

The soil oxygen content eventually stabilized at the user defined set points of 16% and 8%. This took some time to occur for several reasons. Problems with the chemical feed system and the variable speed pumps kept the influent concentration from stabilizing. If the influent concentration is variable, then the microbial activity will be variable and the soil oxygen concentration will not stabilize. Another reason the soil oxygen concentration took a long time to stabilize is because it took a long time for the microbial population to lower the soil oxygen concentration down to the user defined set points. Equipment problems caused the system to be shut down, which allowed the soil oxygen concentration to increase back up to levels near saturation. Once the system was restarted, the microorganisms had to grow again. It takes a significant amount of time to drop the soil oxygen concentration to the levels at which we were studying. Once the equipment had been modified and the system had been left on for approximately two months, the soil oxygen concentrations began to fall and then converged to the user-defined set points.

However, once the soil oxygen concentrations did finally converge, there was no difference in the amount of degradation between the columns that were set at 16% and the columns that were set at 8%. The ethanol passing through each of the eight columns degraded to a level below the limit of detection for the GC. This was expected because ethanol is a small, soluble straight chain organic; making it very easy to be broken down.

Conclusion

This thesis study showed that useful information about the degradation of fuel oxygenates in a laboratory soil column system could be gathered through tracer tests using a YSI conductivity meter and gas chromatograph analysis. The soil columns,

constructed as part of this research, were demonstrated to be a good laboratory system that could be used to study aerobic degradation of ethanol in the vadose zone. Further research is required to test other fuel oxygenates to see if they will degrade in this system. Such oxygenate degradation studies will be critical in helping to find a safe alternative to MTBE.

Study Strengths

In this study, we were able to construct and setup a soil column system to measure biodegradation of fuel oxygenates. Getting all the equipment to work properly was the main goal of the effort. This included getting the oxygen sensors to communicate with the computer control system and the control system to communicate with the pumps. It also included making sure the feed system pumped water and chemicals at a constant rate. The chemicals and water were mixed before entering the top of the columns. The oxygen sensor line had to be sealed in order to keep out air from outside the column, which would have interfered with the soil oxygen concentration measurements. All of the pieces had to work with each other to allow the soil column system to function properly.

A GC method for ethanol in water was developed from a standard method, modified until the chromatographs were clear and uniform. The calibration curves had R-squared values of 0.999 or better, indicating that the standards were mixed and the method worked properly.

The most significant strength of this effort was that the system was able to detect degradation of ethanol. Oxygen sensors showed a decrease in soil oxygen concentration,

which meant biological activity was occurring. Effluent concentrations dropped from the influent concentrations to below the limit of detection for the GC.

Study Limitations

A methodology for the tracer tests was developed; however, the results were not ideal. The breakthrough curves for Columns 5 through 8 included an extended tailing. The results were adjusted and interpolated, which improved them significantly. However, the modified results depend on the assumptions I made when I conditioned the data.

Initially, the influent ethanol concentrations were not consistent and did not stabilize at a constant concentration. Once modifications were made, the influent concentrations still did not stabilize at a constant concentration, though they were in a range that made sense.

The most significant limitation of this effort was the fact that it involved the use of ethanol, which has been found to be biodegradable under a variety of conditions (Mormile *et al.*, 1994). Since it is so easily degraded, I was not able to see differences in the degradation at the different soil oxygen concentrations.

Recommendations for Equipment Improvement

The current equipment configuration could use improvement. The care and maintenance requirements were significant. At first, the most time consuming part of operations was keeping feed water and chemical reservoirs stocked, as well as disposing of the effluent. The fixed speed pumps emptied one 5-gallon bucket every ten to eleven hours, requiring emptying the waste buckets and filling of the reservoirs. This allowed

the equipment to run continuously and keep the microbial population stable. However, once the soil oxygen concentration converged to the user defined set points, the time in which the pumps consumed a bucket of water doubled. The waste buckets still had to be emptied and the reservoir buckets still had to be filled. Automation of the disposal of waste water and filling the reservoir buckets would be very helpful.

Another problem was with the chemical reservoirs. Two eight liter Tedlar bags originally contained the ethanol, each bag feeding four columns. This setup had many problems. The line coming from the bag had to have three "T's" to connect four columns. This allowed for multiple breaks in the line where air could enter the system and modify the flow rate.

After this problem was discovered, 40 mL bottles capped with a Teflon-lined septum and plastic screw tops were used for each column, eliminating air entering the system. However, this created a new problem, the variable speed pumps went through 40 mL in about a day. They continuously needed to be refilled. Once the soil oxygen concentrations converged to the set points, the rate at which the bottles emptied considerably decreased. A recommendation is to find a container that will be large enough to keep from having to refill it too often, yet doesn't allow air into the system or the chemicals to volatilize or leak out.

Recommendations for Further Study

1. Re-run the tracer tests to try and replicate the results that I received. This is important to see if the data are reproducible and if the same breakthrough characteristics are found.

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2. Use a model to simulate the characteristics that were identified in the soil

    column system. The model could simulate microbial growth along the

    column over time, ethanol degradation, etc. It could also incorporate the lag

    time to degrade ethanol, what happened when the system was shut down, etc.

3. Determine a method to quantify the mass of the ethanol fed into each column.

    Once the removal rates are determined for a specified set point, determine

    what is happening to the ethanol that is being degraded. Is it turning into

    daughter products that can be detected by a GC/MS? Is it breaking down all

    the way to carbon dioxide and water?

4. Test alternative fuel oxygenates (ETBE, TAME, and DIPE) that are not

    currently being used in gasoline, as a replacement MTBE because of its

    potential hazards. Determine if ETBE, TAME, and DIPE degrade similarly to

    ethanol under the same conditions. Also, how to their degradation rates,

    compare to those of MTBE found in the literature? Determine if there is a

    significant difference in the rate or extent of degradation at different soil

    oxygen concentrations in the columns.

5. Identify the microorganisms that are degrading the fuel oxygenates. Once the

    apparatus has proven that it can degrade the fuel oxygenates, a culture of the

    microorganisms can be grown and identified. It may be of importance to

know which microorganisms will grow naturally in sandy soil under the

conditions that are created in the lab.

Appendix A: Pump Flow Rates

Fixed Speed Pump

Column	Flow Rate (mL/min)						
	15-Oct	20-Oct	21-Oct	1-Nov	2-Nov	7-Dec	8-Dec
1						26.43	29.55
2	69.13	68.29	67.61	23.33	27.00	28.10	26.97
3						28.10	28.18
4						26.19	27.27
5						26.90	27.27
6						27.14	29.09
7						26.67	28.79
8						26.67	28.48

Variable Speed Pump (Minimum Speed)

Column	Flow Rate (mL/min)				
	21-Oct	21-Nov	7-Dec	8-Dec	9-Dec
1		0.020	0.031	0.029	0.027
2	0.043	0.030	0.031	0.032	0.030
3		0.023	0.026	0.034	0.032
4		0.019	0.013	0.029	0.027
5		0.027	0.032	0.033	0.031
6		0.023	0.016	0.049	0.042
7		0.024	0.038	0.037	0.034
8		0.019	0.035	0.033	0.031

Variable Speed Pump (Maximum Speed)

Column	Flow Rate (mL/min)			
	15-Oct	20-Oct	15-Nov	16-Nov
1			0.26	0.30
2	0.27	0.24	0.10	0.27
3			0.00	0.27
4			0.23	0.24
5			0.28	
6			0.24	
7			0.25	
8			0.26	

Appendix B: Calculated Ethanol Influent Concentrations and Calculations

Column	Concentration (mg/L)
1	919.7
2	865.1
3	725.7
4	389.5
5	932.7
6	462.5
7	1116.9
8	1028.8

Calculated Influent EtOH Concentrations Using 7 December 2003 Flow Rates

Column 1

$$Q_{1a} := 26.43 \cdot \frac{mL}{min} \qquad Q_{2a} := .031 \cdot \frac{mL}{min}$$

$$C_{1a} := 0 \cdot \frac{gm}{mL} \qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1a} \cdot C_{1a} + Q_{2a} \cdot \rho = (Q_{1a} + Q_{2a}) \cdot C_{outa}$$

$$C_{outa} := \frac{Q_{1a} \cdot C_{1a} + Q_{2a} \cdot \rho}{Q_{1a} + Q_{2a}} \qquad C_{outa} = 919.655 \frac{mg}{L}$$

Column 2

$$Q_{1b} := 28.10 \cdot \frac{mL}{min} \qquad Q_{2b} := .031 \cdot \frac{mL}{min}$$

$$C_{1b} := 0 \cdot \frac{gm}{mL} \qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1b} \cdot C_{1b} + Q_{2b} \cdot \rho = (Q_{1b} + Q_{2b}) \cdot C_{outb}$$

$$C_{outb} := \frac{Q_{1b} \cdot C_{1b} + Q_{2b} \cdot \rho}{Q_{1b} + Q_{2b}} \qquad C_{outb} = 865.06 \frac{mg}{L}$$

Column 3

$$Q_{1c} := 28.10 \cdot \frac{mL}{min} \qquad Q_{2c} := .026 \cdot \frac{mL}{min}$$

$$C_{1c} := 0 \cdot \frac{gm}{mL} \qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1c} \cdot C_{1c} + Q_{2c} \cdot \rho = (Q_{1c} + Q_{2c}) \cdot C_{outc}$$

$$C_{outc} := \frac{Q_{1c} \cdot C_{1c} + Q_{2c} \cdot \rho}{Q_{1c} + Q_{2c}} \qquad C_{outc} = 725.663 \frac{mg}{L}$$

Column 4

$$Q_{1d} := 26.19 \cdot \frac{mL}{min} \qquad Q_{2d} := .013 \cdot \frac{mL}{min}$$

$$C_{1d} := 0 \cdot \frac{gm}{mL} \qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1d} \cdot C_{1d} + Q_{2d} \cdot \rho = (Q_{1d} + Q_{2d}) \cdot C_{outd}$$

$$C_{outd} := \frac{Q_{1d} \cdot C_{1d} + Q_{2d} \cdot \rho}{Q_{1d} + Q_{2d}} \qquad C_{outd} = 389.459 \frac{mg}{L}$$

Column 5

$$Q_{1e} := 26.90 \cdot \frac{mL}{min} \qquad\qquad Q_{2e} := .032 \cdot \frac{mL}{min}$$

$$C_{1e} := 0 \cdot \frac{gm}{mL} \qquad\qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1e} \cdot C_{1e} + Q_{2e} \cdot \rho = \left(Q_{1e} + Q_{2e}\right) \cdot C_{oute}$$

$$C_{oute} := \frac{Q_{1e} \cdot C_{1e} + Q_{2e} \cdot \rho}{Q_{1e} + Q_{2e}} \qquad C_{oute} = 932.719 \frac{mg}{L}$$

Column 6

$$Q_{1f} := 27.14 \cdot \frac{mL}{min} \qquad\qquad Q_{2f} := .016 \cdot \frac{mL}{min}$$

$$C_{1f} := 0 \cdot \frac{gm}{mL} \qquad\qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1f} \cdot C_{1f} + Q_{2f} \cdot \rho = \left(Q_{1f} + Q_{2f}\right) \cdot C_{outf}$$

$$C_{outf} := \frac{Q_{1f} \cdot C_{1f} + Q_{2f} \cdot \rho}{Q_{1f} + Q_{2f}} \qquad C_{outf} = 462.513 \frac{mg}{L}$$

Column 7

$$Q_{1g} := 26.67 \cdot \frac{mL}{min} \qquad\qquad Q_{2g} := .038 \cdot \frac{mL}{min}$$

$$C_{1g} := 0 \cdot \frac{gm}{mL} \qquad\qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1g} \cdot C_{1g} + Q_{2g} \cdot \rho = \left(Q_{1g} + Q_{2g}\right) \cdot C_{outg}$$

$$C_{outg} := \frac{Q_{1g} \cdot C_{1g} + Q_{2g} \cdot \rho}{Q_{1g} + Q_{2g}} \qquad C_{outg} = 1116.894 \frac{mg}{L}$$

Column 8

$$Q_{1h} := 26.67 \cdot \frac{mL}{min} \qquad\qquad Q_{2h} := .035 \cdot \frac{mL}{min}$$

$$C_{1h} := 0 \cdot \frac{gm}{mL} \qquad\qquad \rho := 0.785 \cdot \frac{gm}{mL}$$

$$Q_{1h} \cdot C_{1h} + Q_{2h} \cdot \rho = \left(Q_{1h} + Q_{2h}\right) \cdot C_{outh}$$

$$C_{outh} := \frac{Q_{1h} \cdot C_{1h} + Q_{2h} \cdot \rho}{Q_{1h} + Q_{2h}} \qquad C_{outh} = 1028.834 \frac{mg}{L}$$

Appendix C: Conductivity Probe Calibration Curves

Calibration Curve Conducted Prior to Tracer Tests Using De-Ionized Water

Conductivity (µS/cm)	Concentration (mg/L)
1037	500
2248	1000
3047	1500
4177	2000

Calibration Curve

$y = 0.4775x$

$R^2 = 0.994$

Concentration (mg/L) vs. Conductivity (µS/cm)
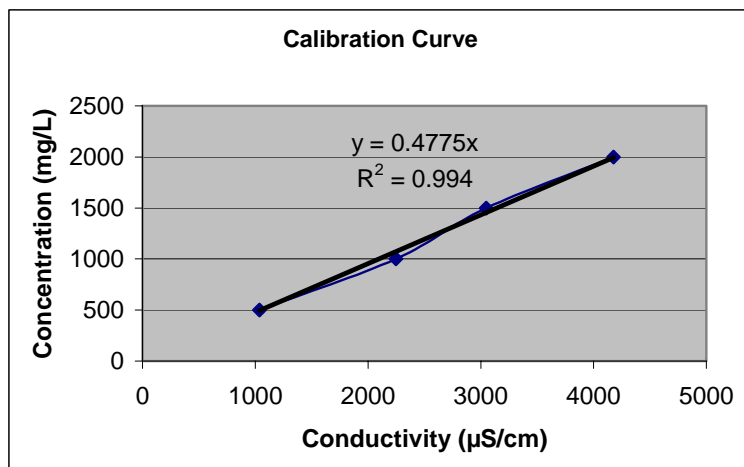
Calibration Curve Conducted After Tracer Tests Using De-Ionized Water

Conductivity (µS/cm)	Concentration (mg/L)
1090	500
2100	1000
3082	1500
4108	2000

Calibration Curve

$y = 0.4843x$

$R^2 = 0.999$

Concentration (mg/L) vs. Conductivity (µS/cm)
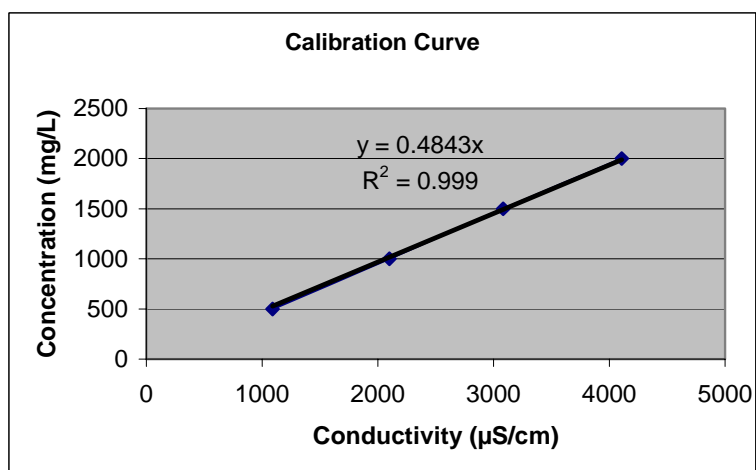
Conductivity Curve Conducted After Tracer Tests Using Tap Water

Conductivity (µS/cm)	Concentration (mg/L)
848	0
860	10
1004	100
1686	500
2589	1000
3558	1500
4563	2000

Calibration Curve

$y = 0.5413x - 438.35$

$R^2 = 0.999$

Concentration (mg/L) vs. Conductivity (µS/cm)
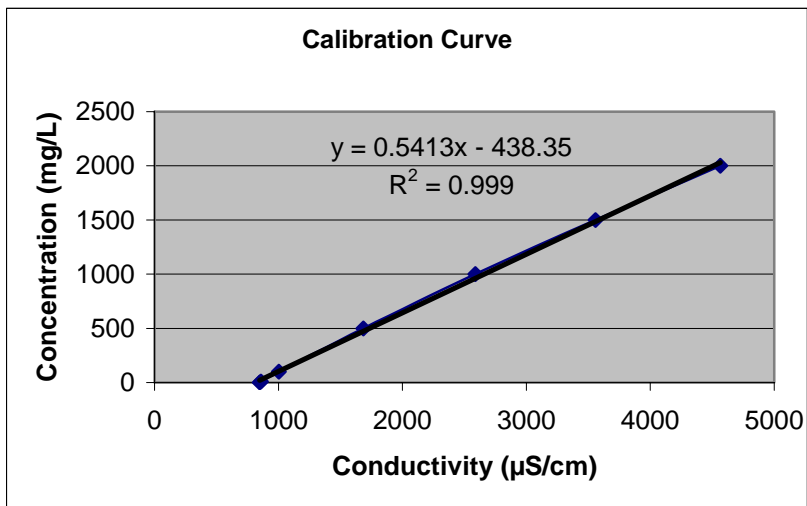
Appendix D: Column 1 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3332	1365.3

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Relative Concentration
0	861	27.7	0.02
76	838	15.3	0.01
121	824	7.7	0.01
216	821	6.1	0.00
266	834	13.1	0.01
328	827	9.3	0.01
440	841	16.9	0.01
464	1000	103.0	0.08
494	1273	250.7	0.18
560	1840	557.6	0.41
602	2138	718.9	0.53
638	2380	849.9	0.62
698	2633	986.9	0.72
740	2806	1080.5	0.79
788	2877	1119.0	0.82
842	2964	1166.1	0.85
902	2989	1179.6	0.86
973	3096	1237.5	0.91
1044	3091	1234.8	0.90
1113	3105	1242.4	0.91
1175	3108	1244.0	0.91
1232	2650	996.1	0.73
1296	2057	675.1	0.49
1358	1562	407.2	0.30
1416	1249	237.7	0.17
1480	1100	157.1	0.12
1765	895	46.1	0.03
2063	856	25.0	0.02

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
935159.3815	920221571.8	643.49	26.43	24.7	17.01

10.7 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1365.3	681.1	929854	26.43	24.6	-0.57

78

79
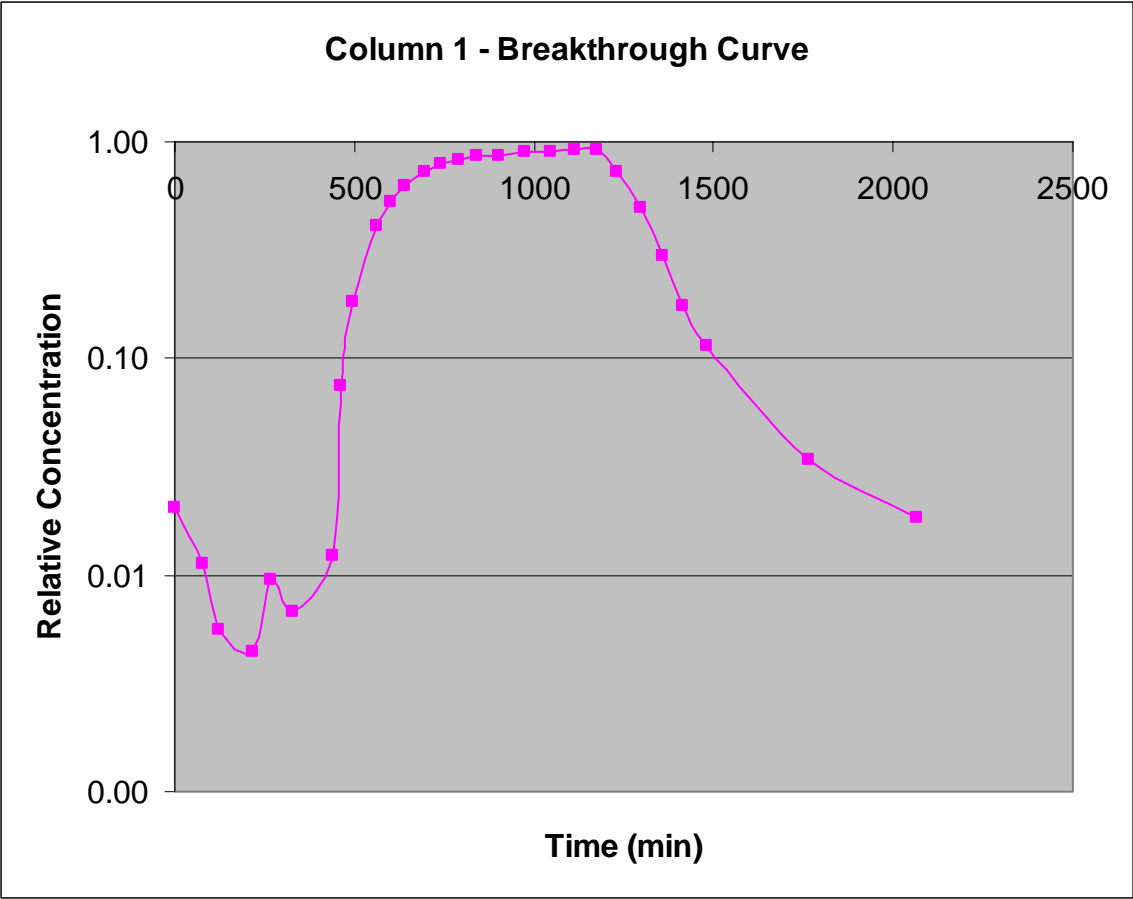
Appendix E: Column 2 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3280	1337.1

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Relative Conc
0	851	22.3	0.02
76	852	22.8	0.02
121	849	21.2	0.02
216	847	20.1	0.02
266	861	27.7	0.02
328	843	18.0	0.01
440	1454	348.7	0.26
454	1757	512.7	0.38
465	1870	573.9	0.43
495	2114	706.0	0.53
561	2514	922.5	0.69
604	2709	1028.0	0.77
639	2850	1104.4	0.83
699	2942	1154.2	0.86
742	3017	1194.8	0.89
790	3057	1216.4	0.91
843	3063	1219.7	0.91
903	3102	1240.8	0.93
975	3116	1248.3	0.93
1046	2542	937.6	0.70
1114	1976	631.3	0.47
1177	1606	431.0	0.32
1233	1311	271.3	0.20
1297	1186	203.6	0.15
1360	1145	181.4	0.14
1418	1005	105.7	0.08
1481	958	80.2	0.06
1766	887	41.8	0.03
2064	850	21.8	0.02
2765	837	14.7	0.01

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
861323	757111346.1	558.67	28.10	24.2	15.70

9.3 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1337.1	640.7	856659	28.10	24.1	-0.54

Column 2 - Breakthrough Curve
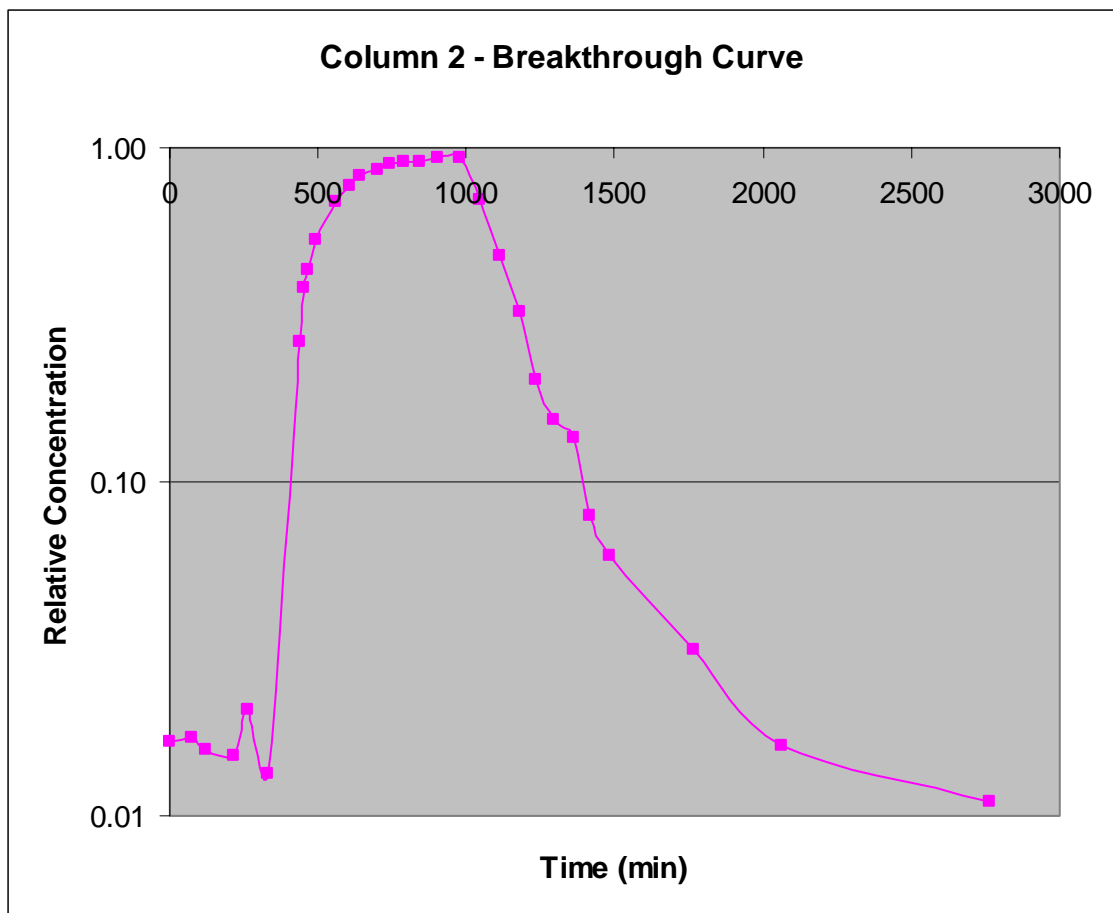
Appendix F: Column 3 Tracer Test Results

Initial Conductivity (µS/cm)	Concentration (mg/L)
3351	1375.5

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	823	7.1	0.01
76	813	1.7	0.00
121	812	1.2	0.00
216	828	9.8	0.01
266	825	8.2	0.01
328	830	10.9	0.01
440	849	21.2	0.02
467	1123	169.5	0.12
496	1455	349.2	0.25
563	2011	650.2	0.47
605	2247	778.0	0.57
640	2431	877.6	0.64
701	2638	989.6	0.72
743	2785	1069.2	0.78
791	2878	1119.5	0.81
845	2958	1162.8	0.85
904	3037	1205.6	0.88
976	3103	1241.3	0.90
1047	3079	1228.3	0.89
1115	2721	1034.5	0.75
1178	2041	666.4	0.48
1235	1592	423.4	0.31
1299	1337	285.4	0.21
1361	1214	218.8	0.16
1419	1120	167.9	0.12
1482	1065	138.1	0.10
1767	908	53.2	0.04
2066	845	19.0	0.01

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
822298	776303187	623.73	28.10	23.1	17.52

10.4 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1375.5	640.68	881282	28.10	24.8	6.69

Column 3 - Breakthrough Curve
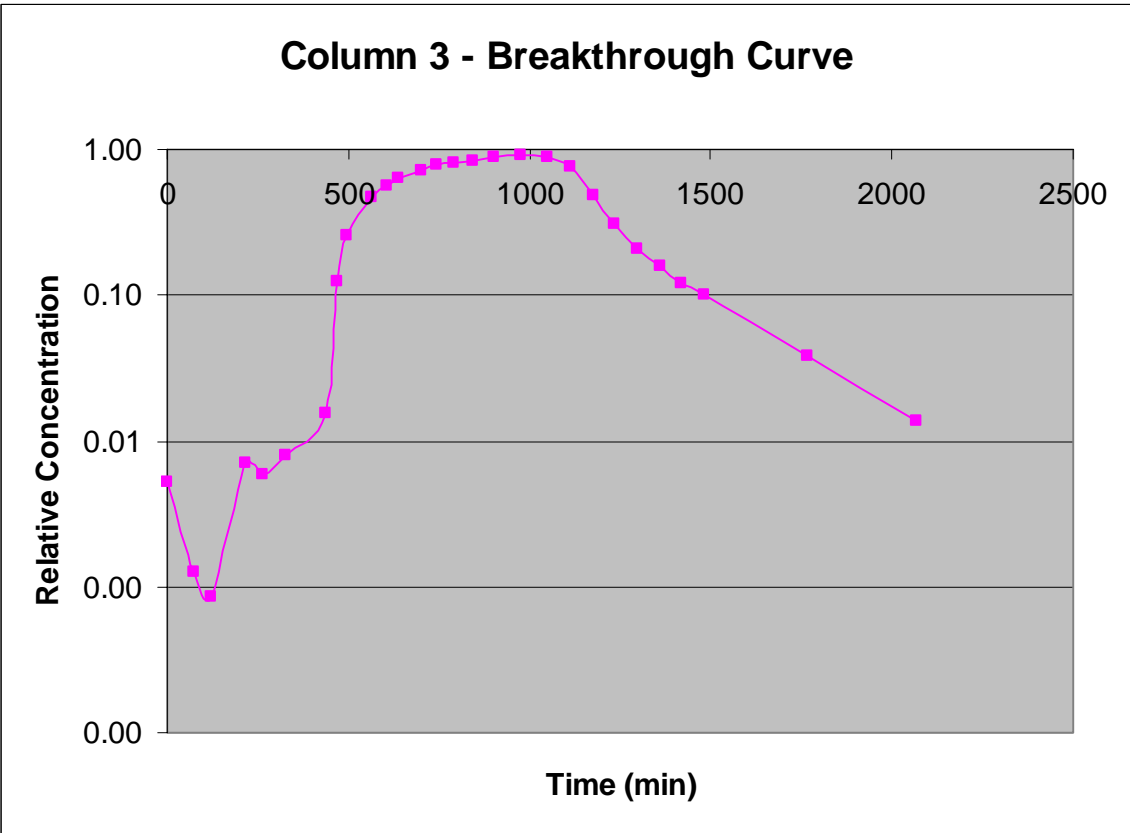
Appendix G: Column 4 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3276	1334.9

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	827	9.3	0.007
76	826	8.8	0.007
121	810	0.1	0.000
216	814	2.3	0.002
266	820	5.5	0.004
328	818	4.4	0.003
440	821	6.1	0.005
468	831	11.5	0.009
497	946	73.7	0.055
564	1350	292.4	0.219
607	1708	486.2	0.364
641	1938	610.7	0.457
702	2362	840.2	0.629
745	2540	936.6	0.702
792	2725	1036.7	0.777
846	2874	1117.3	0.837
906	2973	1170.9	0.877
977	2996	1183.4	0.886
1048	2978	1173.6	0.879
1117	3067	1221.8	0.915
1179	3058	1216.9	0.912
1236	2986	1178.0	0.882
1300	2545	939.3	0.704
1362	1872	575.0	0.431
1420	1428	334.6	0.251
1483	1141	179.3	0.134
1768	932	66.1	0.050
2067	894	45.6	0.034
2767	851	22.3	0.017
2902	835	13.6	0.010

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
935716	1011730826	737.60	26.19	24.5	19.32

12.3 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1334.9	687.27	917474	26.19	24.0	-1.99

Column 4 - Breakthrough Curve
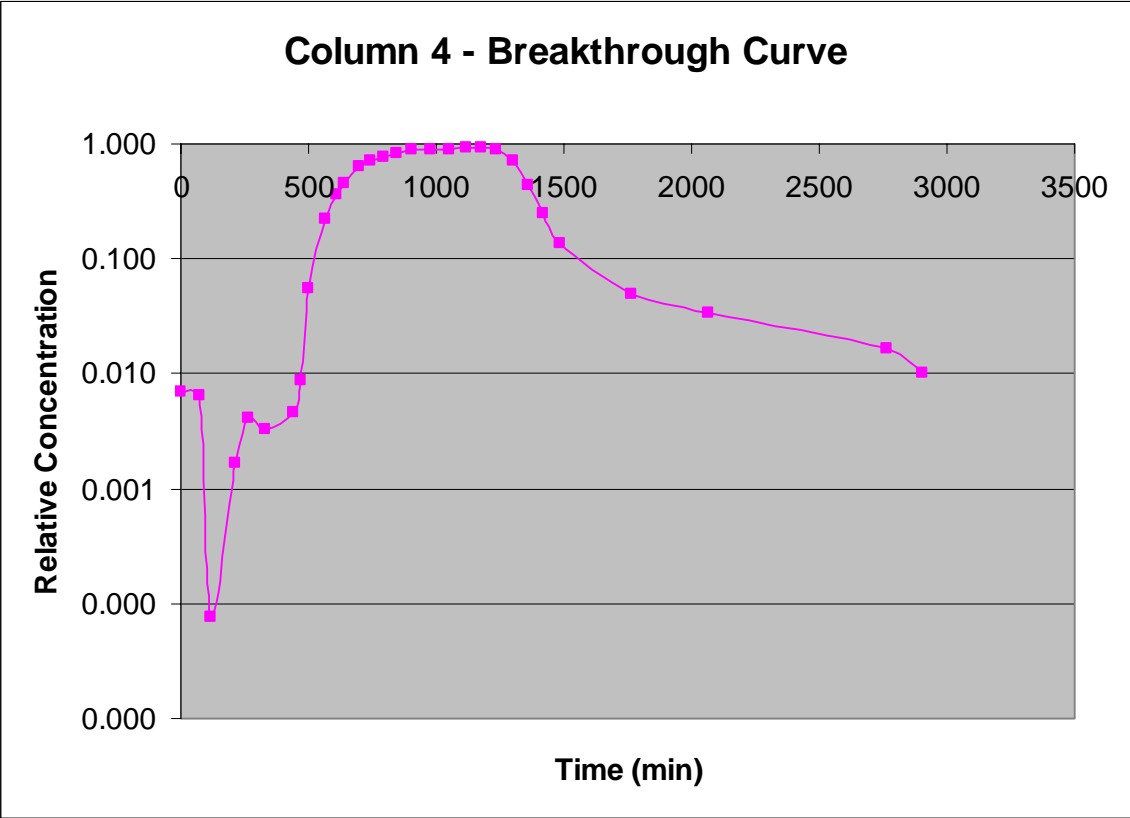
Appendix H: Column 5 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3278	1336.0

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	914	56.4	0.042
76	920	59.6	0.045
121	913	55.9	0.042
216	923	61.3	0.046
266	929	64.5	0.048
328	940	70.5	0.053
440	927	63.4	0.047
470	940	70.5	0.053
499	985	94.8	0.071
565	1342	288.1	0.216
608	1625	441.3	0.330
642	1859	567.9	0.425
703	2240	774.2	0.579
746	2458	892.2	0.668
793	2650	996.1	0.746
848	2825	1090.8	0.816
907	2959	1163.4	0.871
978	3074	1225.6	0.917
1049	3103	1241.3	0.929
1118	3182	1284.1	0.961
1180	3212	1300.3	0.973
1237	3111	1245.6	0.932
1301	2796	1075.1	0.805
1364	2157	729.2	0.546
1421	1666	463.5	0.347
1484	1442	342.2	0.256
1769	1135	176.0	0.132
2068	1117	166.3	0.124
2768	1141	179.3	0.134
2904	1219	221.5	0.166
3393	856	25.0	0.019

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1258444	1698303519	1015.01	26.90	33.9	27.31

16.9 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1336.0	669.03	893840	26.90	24.0	-40.79

Column 5 - Breakthrough Curve

Appendix I: Column 6 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3325	1361.5

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	860	27.2	0.020
76	863	28.8	0.021
121	852	22.8	0.017
216	867	31.0	0.023
266	874	34.7	0.026
328	863	28.8	0.021
440	864	29.3	0.022
472	948	74.8	0.055
500	1128	172.2	0.127
566	1531	390.4	0.287
609	1882	580.4	0.426
643	2121	709.7	0.521
704	2426	874.8	0.643
747	2645	993.4	0.730
795	2785	1069.2	0.785
849	2892	1127.1	0.828
908	2986	1178.0	0.865
980	3040	1207.2	0.887
1051	3103	1241.3	0.912
1119	3115	1247.8	0.917
1181	3076	1226.7	0.901
1238	2792	1073.0	0.788
1303	2210	757.9	0.557
1365	1778	524.1	0.385
1423	1435	338.4	0.249
1486	1252	239.4	0.176
1770	1062	136.5	0.100
2069	956	79.1	0.058
2769	923	61.3	0.045
2907	971	87.3	0.064
3395	872	33.7	0.025
3595	831	11.5	0.008

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1065645	1270598104	860.75	27.14	28.9	23.36

14.3 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1361.5	663.2	902871	27.14	24.5	-18.03

Column 6 - Breakthrough Curve
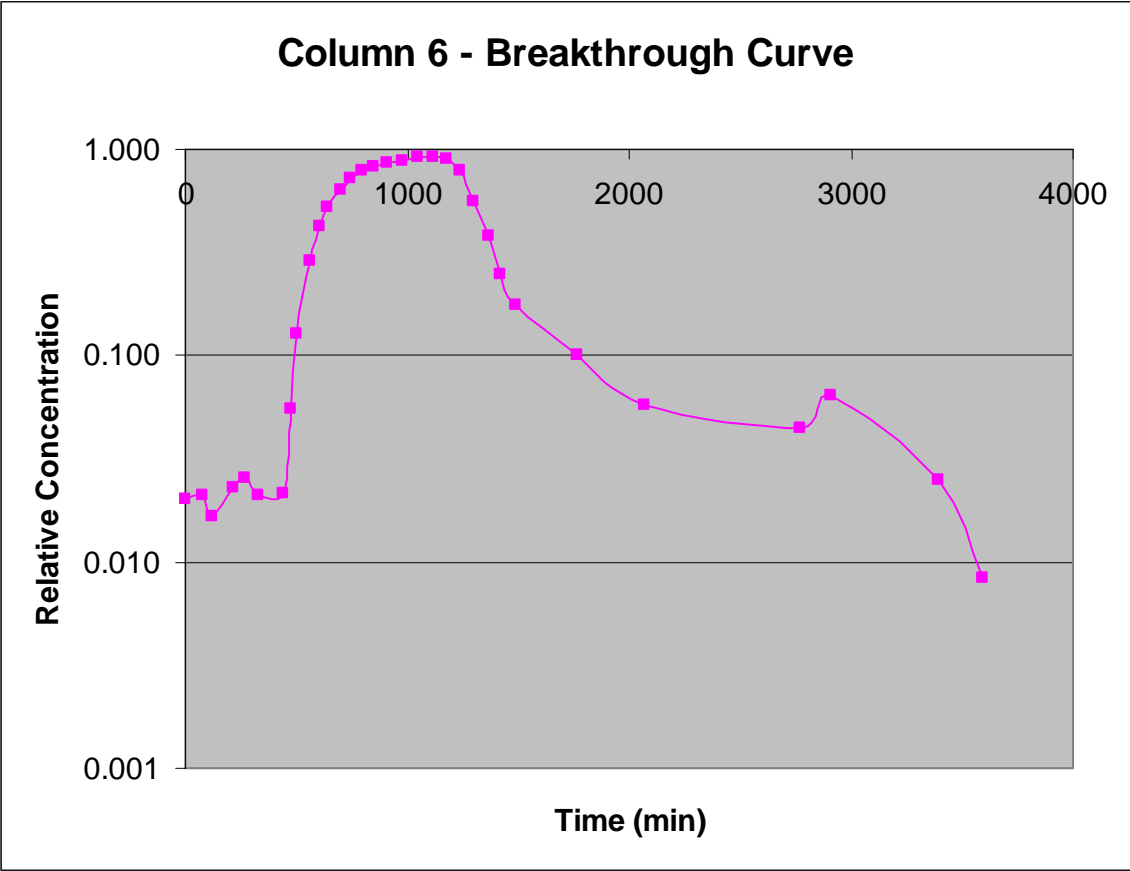
Appendix J: Column 7 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3324	1360.9

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	893	45.0	0.033
76	881	38.5	0.028
121	870	32.6	0.024
216	864	29.3	0.022
266	866	30.4	0.022
328	853	23.4	0.017
440	854	23.9	0.018
473	926	62.9	0.046
502	1059	134.9	0.099
567	1426	333.5	0.245
610	1756	512.2	0.376
644	1948	616.1	0.453
705	2264	787.2	0.578
748	2464	895.4	0.658
796	2679	1011.8	0.743
850	2819	1087.6	0.799
909	2953	1160.1	0.852
981	3028	1200.7	0.882
1052	3150	1266.7	0.931
1121	3187	1286.8	0.946
1183	3162	1273.2	0.936
1240	2950	1158.5	0.851
1304	2321	818.0	0.601
1367	1866	571.7	0.420
1424	1533	391.5	0.288
1487	1320	276.2	0.203
1771	1118	166.8	0.123
2070	1103	158.7	0.117
2770	1092	152.7	0.112
2912	1146	182.0	0.134
3397	926	62.9	0.046
3596	861	27.7	0.020
4306	815	2.8	0.002

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1206578	1663691718	1041.35	26.67	32.2	27.77

17.4 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1360.9	675	918629	26.67	24.5	-31.35

Column 7 - Breakthrough Curve

Relative Concentration
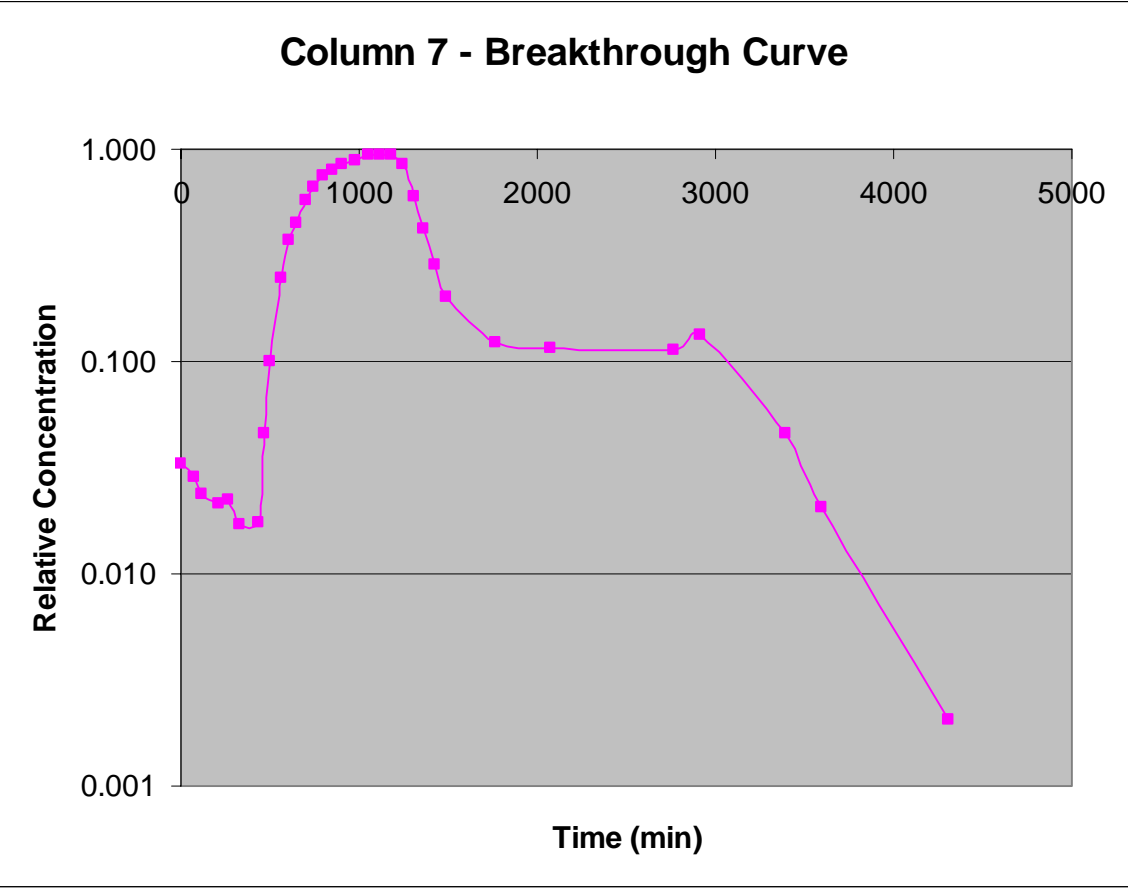
Time (min)

Appendix K: Column 8 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3485	1448.1

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc
0	913	55.9	0.039
76	909	53.7	0.037
121	906	52.1	0.036
216	930	65.1	0.045
266	929	64.5	0.045
328	914	56.4	0.039
440	1000	103.0	0.071
474	1191	206.3	0.142
503	1390	314.1	0.217
568	1874	576.0	0.398
611	2179	741.1	0.512
645	2371	845.1	0.584
706	2610	974.4	0.673
749	2784	1068.6	0.738
798	2898	1130.3	0.781
852	2951	1159.0	0.800
911	3052	1213.7	0.838
982	3171	1278.1	0.883
1053	3220	1304.6	0.901
1122	3266	1329.5	0.918
1184	3251	1321.4	0.913
1241	3020	1196.4	0.826
1306	2393	857.0	0.592
1368	1920	600.9	0.415
1425	1571	412.0	0.285
1488	1410	324.9	0.224
1772	1044	126.8	0.088
2071	1004	105.1	0.073
2771	992	98.6	0.068
2914	1022	114.9	0.079
3399	884	40.2	0.028
3597	848	20.7	0.014
4307	825	8.2	0.006

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1238357	1526491612	895.17	26.67	33.0	23.87

14.9 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1448.1	675	977454	26.67	26.1	-26.69

Column 8 - Breakthrough Curve
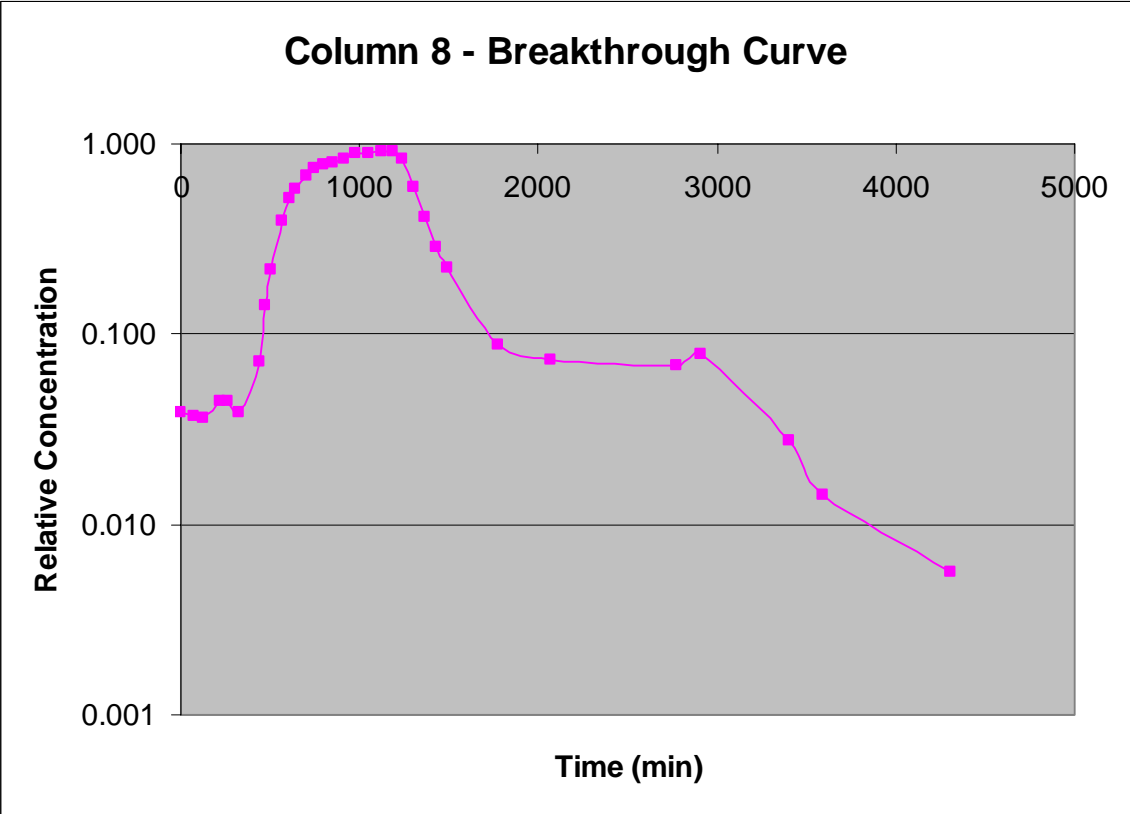
Appendix L: Formulas for Tracer Test Results

	A	B	C	D	E	F
1	Initial Conductivity (µS/cm)	Concentration (mg/L)				
2	3332	=0.5413*A2-438.35				
3						
4	Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Relative Concentration		
5	0	861	=0.5413*B5-438.35	=C5/$B$2		
6	76	838	=0.5413*B6-438.35	=C6/$B$2		
7	121	824	=0.5413*B7-438.35	=C7/$B$2		
8	216	821	=0.5413*B8-438.35	=C8/$B$2		
9	266	834	=0.5413*B9-438.35	=C9/$B$2		
10	328	827	=0.5413*B10-438.35	=C10/$B$2		
11	440	841	=0.5413*B11-438.35	=C11/$B$2		
12	464	1000	=0.5413*B12-438.35	=C12/$B$2		
13	494	1273	=0.5413*B13-438.35	=C13/$B$2		
14	560	1840	=0.5413*B14-438.35	=C14/$B$2		
15	602	2138	=0.5413*B15-438.35	=C15/$B$2		
16	638	2380	=0.5413*B16-438.35	=C16/$B$2		
17	698	2633	=0.5413*B17-438.35	=C17/$B$2		
18	740	2806	=0.5413*B18-438.35	=C18/$B$2		
19	788	2877	=0.5413*B19-438.35	=C19/$B$2		
20	842	2964	=0.5413*B20-438.35	=C20/$B$2		
21	902	2989	=0.5413*B21-438.35	=C21/$B$2		
22	973	3096	=0.5413*B22-438.35	=C22/$B$2		
23	1044	3091	=0.5413*B23-438.35	=C23/$B$2		
24	1113	3105	=0.5413*B24-438.35	=C24/$B$2		
25	1175	3108	=0.5413*B25-438.35	=C25/$B$2		
26	1232	2650	=0.5413*B26-438.35	=C26/$B$2		
27	1296	2057	=0.5413*B27-438.35	=C27/$B$2		
28	1358	1562	=0.5413*B28-438.35	=C28/$B$2		
29	1416	1249	=0.5413*B29-438.35	=C29/$B$2		
30	1480	1100	=0.5413*B30-438.35	=C30/$B$2		
31	1765	895	=0.5413*B31-438.35	=C31/$B$2		
32	2063	856	=0.5413*B32-438.35	=C32/$B$2		
33						
34						
35						
36	Output					
37	Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
38	=Moment0(5,32,1,3)	=Moment1(5,32,1,3)	=(B38/A38)-(B42/2)	=I:\My Documents\AFIT\Thesis\Data\Appendix A Pump flow rates.xls[Flow Rates]$H$5	=A38*D38/1000/1000	=D38*C38/1000
39			=C38/60			
40	Input					
41	Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
42	=B2	=18000/D38	=A42*B42	=D38	=D42*C42/1000/1000	=100*(E42-E38)/E42

Appendix M: Column Retention Times, Pore Volumes, and Mass Balance Error

Original Data

Column	Retention Time (hr)	Pore Volume (L)	Mass Balance Error (%)
1	10.7	17.0	-0.57
2	9.3	15.7	-0.54
3	10.4	17.5	6.69
4	12.3	19.3	-1.99
5	16.9	27.3	-40.79
6	14.3	23.4	-18.03
7	17.4	27.8	-31.35
8	14.9	23.9	-26.69

Appendix N: Adjusted Column 5 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3278	1336.0

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc	Interpolated and base line adjusted Rel Conc
0	914	56.4	0.042	
76	920	59.6	0.045	0.003
121	913	55.9	0.042	0.000
216	923	61.3	0.046	0.004
266	929	64.5	0.048	0.006
328	940	70.5	0.053	0.011
440	927	63.4	0.047	0.005
470	940	70.5	0.053	0.011
499	985	94.8	0.071	0.029
565	1342	288.1	0.216	0.174
608	1625	441.3	0.330	0.288
642	1859	567.9	0.425	0.383
703	2240	774.2	0.579	0.537
746	2458	892.2	0.668	0.626
793	2650	996.1	0.746	0.704
848	2825	1090.8	0.816	0.774
907	2959	1163.4	0.871	0.829
978	3074	1225.6	0.917	0.875
1049	3103	1241.3	0.929	0.887
1118	3182	1284.1	0.961	0.919
1180	3212	1300.3	0.973	0.931
1237	3111	1245.6	0.932	0.890
1301	2796	1075.1	0.805	0.763
1364	2157	729.2	0.546	0.504
1421	1666	463.5	0.347	0.305
1484	1442	342.2	0.256	0.214
1769	1135	176.0	0.132	0.090
2068	1117	166.3	0.124	0.04
2768	1141	179.3	0.134	0.007
2904	1219	221.5	0.166	
3393	856	25.0	0.019	

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1258444	1698303519	1015.01	26.90	33.9	27.31

16.9 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1336.0	669.03	893840	26.90	24.0	-40.79

Interpolated Output

Interpolated Zero moment	first moment	Rel First moment	Retention time (hrs)	Pore Vol (L)	Mass Balance Error (%)
708.8	790955	1115.9	13.02	21.0	-5.95

Column 5 - Modified Breakthrough Curve
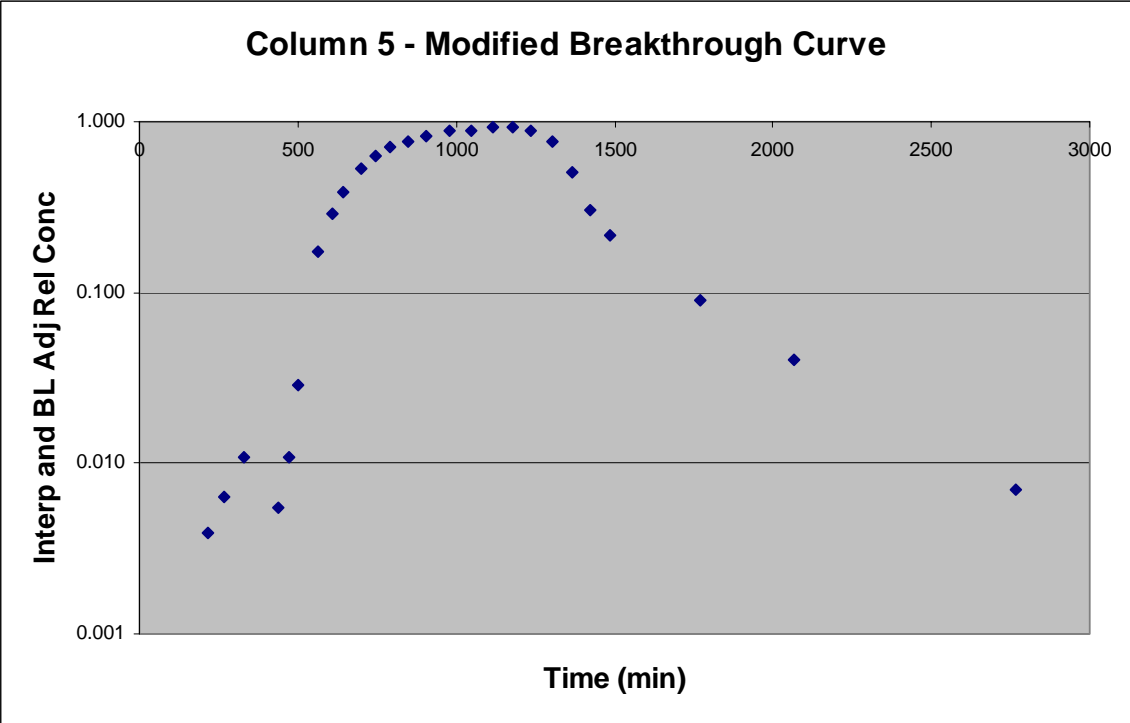
Appendix O: Adjusted Column 6 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3325	1361.5

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc	Interpolated and base line adjusted Rel Conc
0	860	27.2	0.020	
76	863	28.8	0.021	0.001
121	852	22.8	0.017	-0.003
216	867	31.0	0.023	0.003
266	874	34.7	0.026	0.006
328	863	28.8	0.021	0.001
440	864	29.3	0.022	0.002
472	948	74.8	0.055	0.035
500	1128	172.2	0.127	0.107
566	1531	390.4	0.287	0.267
609	1882	580.4	0.426	0.406
643	2121	709.7	0.521	0.501
704	2426	874.8	0.643	0.623
747	2645	993.4	0.730	0.710
795	2785	1069.2	0.785	0.765
849	2892	1127.1	0.828	0.808
908	2986	1178.0	0.865	0.845
980	3040	1207.2	0.887	0.867
1051	3103	1241.3	0.912	0.892
1119	3115	1247.8	0.917	0.897
1181	3076	1226.7	0.901	0.881
1238	2792	1073.0	0.788	0.768
1303	2210	757.9	0.557	0.537
1365	1778	524.1	0.385	0.365
1423	1435	338.4	0.249	0.229
1486	1252	239.4	0.176	0.156
1770	1062	136.5	0.100	0.080
2069	956	79.1	0.058	0.038
2769	923	61.3	0.045	0.010
2907	971	87.3	0.064	0.007
3395	872	33.7	0.025	0.003
3595	831	11.5	0.008	

Output

| Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1065645	1270598104	860.75	27.14	28.9	23.36

14.3 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1361.5	663.1578947	902871	27.14	24.5	-18.03

Interpolated Output

Interpolated Zero moment	first moment	Rel First moment	Retention time (hrs)	Pore Vol (L)	Mass Balance Error (%)
693.2	753774	1087.4	12.60	20.5	-4.53

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Column 6 - Modified Breakthrough Curve
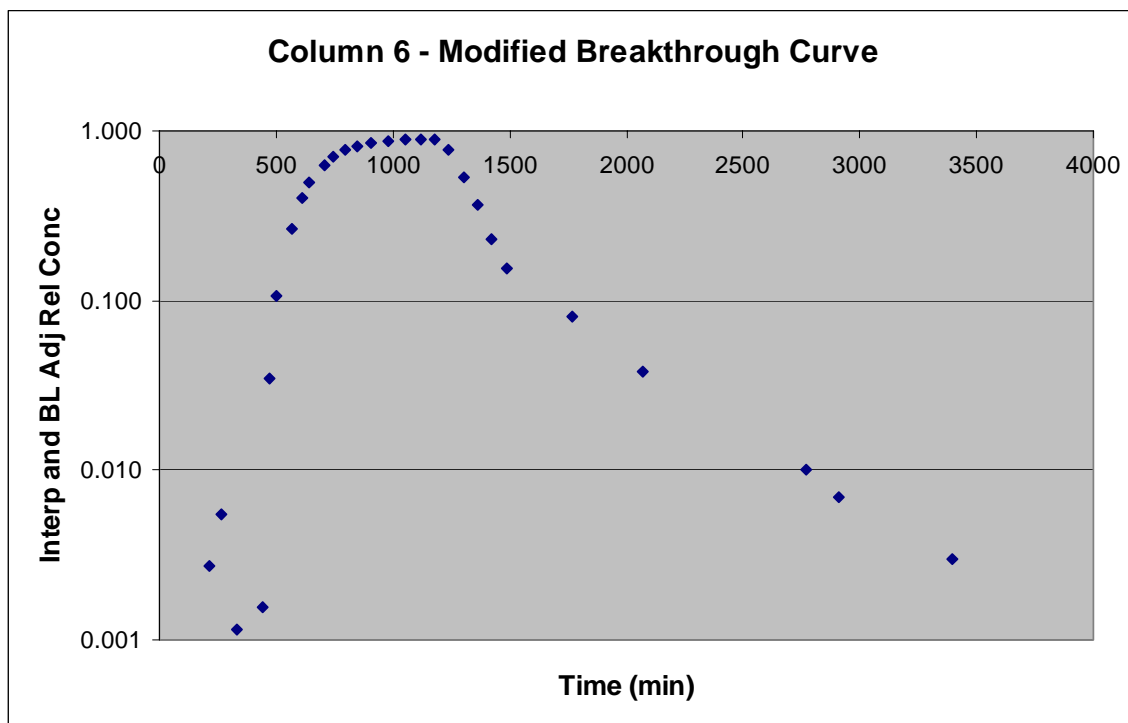
Appendix P: Adjusted Column 7 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3324	1360.9

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc	Interpolated and base line adjusted Rel Conc
0	893	45.0	0.033	
76	881	38.5	0.028	
121	870	32.6	0.024	
216	864	29.3	0.022	
266	866	30.4	0.022	
328	853	23.4	0.017	
440	854	23.9	0.018	
473	926	62.9	0.046	0.013
502	1059	134.9	0.099	0.066
567	1426	333.5	0.245	0.212
610	1756	512.2	0.376	0.343
644	1948	616.1	0.453	0.420
705	2264	787.2	0.578	0.545
748	2464	895.4	0.658	0.625
796	2679	1011.8	0.743	0.710
850	2819	1087.6	0.799	0.766
909	2953	1160.1	0.852	0.819
981	3028	1200.7	0.882	0.849
1052	3150	1266.7	0.931	0.898
1121	3187	1286.8	0.946	0.913
1183	3162	1273.2	0.936	0.903
1240	2950	1158.5	0.851	0.818
1304	2321	818.0	0.601	0.568
1367	1866	571.7	0.420	0.387
1424	1533	391.5	0.288	0.255
1487	1320	276.2	0.203	0.170
1771	1118	166.8	0.123	0.090
2070	1103	158.7	0.117	0.050
2770	1092	152.7	0.112	0.015
2912	1146	182.0	0.134	0.011
3397	926	62.9	0.046	0.005
3596	861	27.7	0.020	
4306	815	2.8	0.002	

Output

Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1206578	1663691718	1041.35	26.67	32.2	27.77

17.4 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1360.9	675	918628.56	26.67	24.5	-31.35

Interpolated Output

Interpolated Zero moment	first moment	Rel First moment	Retention time (hrs)	Pore Vol (L)	Mass Balance Error (%)
690.6	781124	1131.1	13.23	21.2	-2.31

Column 7 - Modified Breakthrough Curve
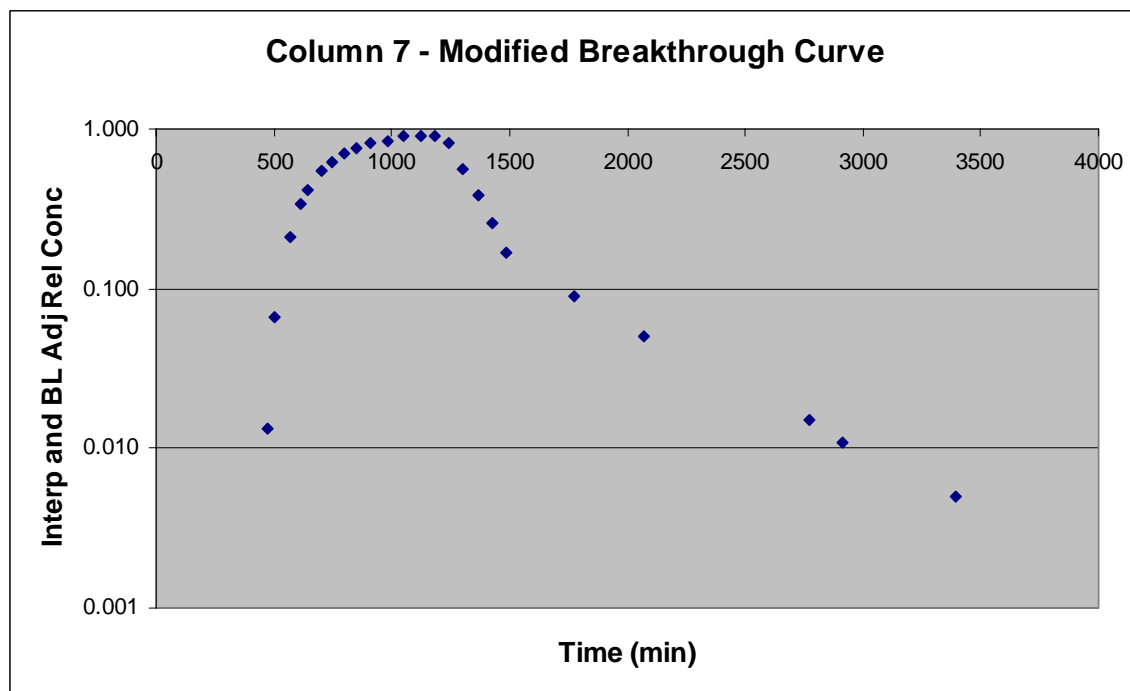
Appendix Q: Adjusted Column 8 Tracer Test Results

Initial Concuctivity (µS/cm)	Concentration (mg/L)
3485	1448.1

Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc	Interpolated and base line adjusted Rel Conc
0	913	55.9	0.039	
76	909	53.7	0.037	
121	906	52.1	0.036	
216	930	65.1	0.045	0.006
266	929	64.5	0.045	0.006
328	914	56.4	0.039	0.000
440	1000	103.0	0.071	0.032
474	1191	206.3	0.142	0.103
503	1390	314.1	0.217	0.178
568	1874	576.0	0.398	0.359
611	2179	741.1	0.512	0.473
645	2371	845.1	0.584	0.545
706	2610	974.4	0.673	0.634
749	2784	1068.6	0.738	0.699
798	2898	1130.3	0.781	0.742
852	2951	1159.0	0.800	0.761
911	3052	1213.7	0.838	0.799
982	3171	1278.1	0.883	0.844
1053	3220	1304.6	0.901	0.862
1122	3266	1329.5	0.918	0.879
1184	3251	1321.4	0.913	0.874
1241	3020	1196.4	0.826	0.787
1306	2393	857.0	0.592	0.553
1368	1920	600.9	0.415	0.376
1425	1571	412.0	0.285	0.246
1488	1410	324.9	0.224	0.185
1772	1044	126.8	0.088	0.049
2071	1004	105.1	0.073	0.022
2771	992	98.6	0.068	0.004
2914	1022	114.9	0.079	0.003
3399	884	40.2	0.028	
3597	848	20.7	0.014	
4307	825	8.2	0.006	

Output

| Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
1238357	1526491612	895.17	26.67	33.0	23.87

14.9 hrs

Input

Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
1448.1	675	977454.3375	26.67	26.1	-26.69

Interpolated Output

Interpolated Zero moment	first moment	Rel First moment	Retention time (hrs)	Pore Vol (L)	Mass Balance Error (%)
681.5	718054	1053.6	11.94	19.1	-0.97

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Column 8 - Modified Breakthrough Curve

Appendix R: Formulas for Adjusted Tracer Test Results

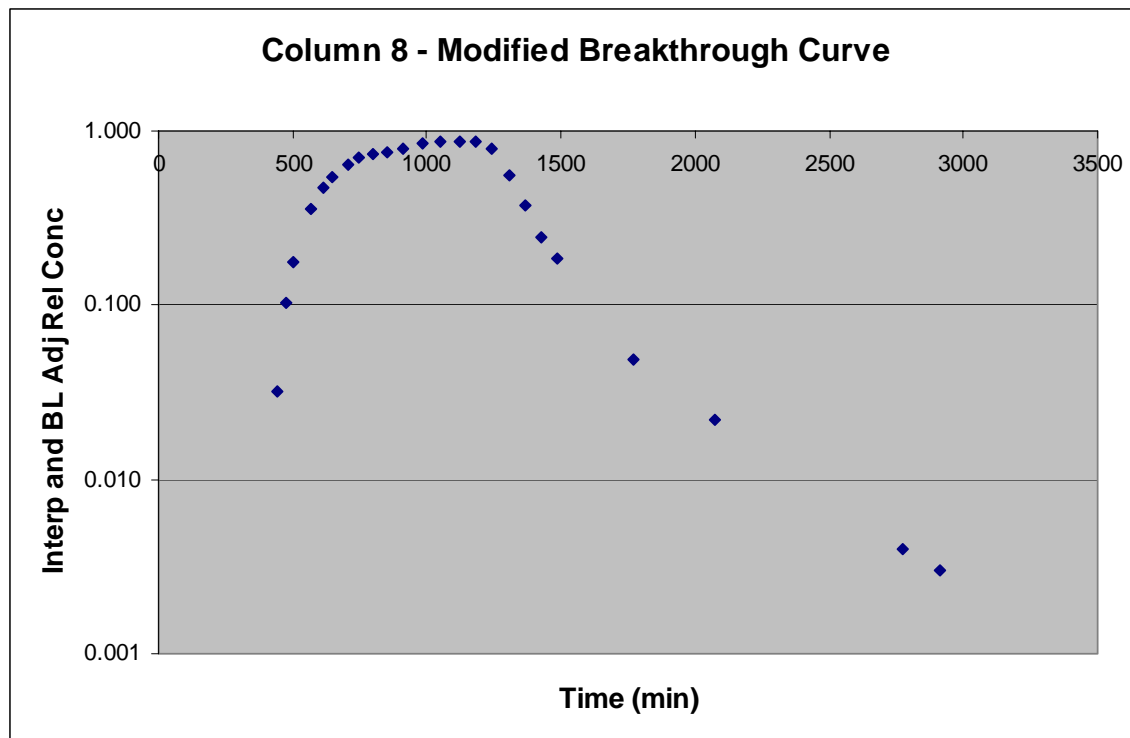| #	A	B	C	D	E	F
1	Initial Concuctivity (µS/cm)	Concentration (mg/L)				
2	3278	=0.5413*A2-438.35				
3						
4	Time (min)	Conductivity (µS/cm)	Concentration (mg/L)	Rel Conc	Interpolated and base line adjusted Rel Conc	
5	0	914	=0.5413*B5-438.35	=C5/$B$2	=D5-0.042	
6	76	920	=0.5413*B6-438.35	=C6/$B$2	=D6-0.042	
7	121	913	=0.5413*B7-438.35	=C7/$B$2	=D7-0.042	
8	216	923	=0.5413*B8-438.35	=C8/$B$2	=D8-0.042	
9	266	929	=0.5413*B9-438.35	=C9/$B$2	=D9-0.042	
10	328	940	=0.5413*B10-438.35	=C10/$B$2	=D10-0.042	
11	440	927	=0.5413*B11-438.35	=C11/$B$2	=D11-0.042	
12	470	940	=0.5413*B12-438.35	=C12/$B$2	=D12-0.042	
13	499	985	=0.5413*B13-438.35	=C13/$B$2	=D13-0.042	
14	565	1342	=0.5413*B14-438.35	=C14/$B$2	=D14-0.042	
15	608	1625	=0.5413*B15-438.35	=C15/$B$2	=D15-0.042	
16	642	1859	=0.5413*B16-438.35	=C16/$B$2	=D16-0.042	
17	703	2240	=0.5413*B17-438.35	=C17/$B$2	=D17-0.042	
18	746	2458	=0.5413*B18-438.35	=C18/$B$2	=D18-0.042	
19	793	2650	=0.5413*B19-438.35	=C19/$B$2	=D19-0.042	
20	848	2825	=0.5413*B20-438.35	=C20/$B$2	=D20-0.042	
21	907	2959	=0.5413*B21-438.35	=C21/$B$2	=D21-0.042	
22	978	3074	=0.5413*B22-438.35	=C22/$B$2	=D22-0.042	
23	1049	3103	=0.5413*B23-438.35	=C23/$B$2	=D23-0.042	
24	1118	3182	=0.5413*B24-438.35	=C24/$B$2	=D24-0.042	
25	1180	3212	=0.5413*B25-438.35	=C25/$B$2	=D25-0.042	
26	1237	3111	=0.5413*B26-438.35	=C26/$B$2	=D26-0.042	
27	1301	2796	=0.5413*B27-438.35	=C27/$B$2	=D27-0.042	
28	1364	2157	=0.5413*B28-438.35	=C28/$B$2	=D28-0.042	
29	1421	1666	=0.5413*B29-438.35	=C29/$B$2	=D29-0.042	
30	1484	1442	=0.5413*B30-438.35	=C30/$B$2	=D30-0.042	
31	1769	1135	=0.5413*B31-438.35	=C31/$B$2	=D31-0.042	
32	2068	1117	=0.5413*B32-438.35	=C32/$B$2	0.04	
33	2768	1141	=0.5413*B33-438.35	=C33/$B$2	0.007	
34	2904	1219	=0.5413*B34-438.35	=C34/$B$2		
35	3393	856	=0.5413*B35-438.35	=C35/$B$2		
36						
37						
38	Output					
39	Zero Moment (mg*min/L)	First Moment	Ret Time (min)	Flow Rate (mL/min)	Mass (g)	Pore Vol (L)
40	=Moment0(5,35,1,3)	=Moment1(5,35,1,3)	=(B40/A40)-(B44/2)	=I:\My Documents\AFIT\Thesis\Data\Appendix A Pump flow rates.xls[Flow Rates]$H$9	=A40*D40/1000/1000	=D40*C40/1000
41	Input		=C40/60			
42	Input					
43	Initial Concentration (mg/L)	Injection Time (min)	Zero Moment (mg*min/L)	Flow Rate (mL/min)	Mass (g)	Mass Balance Error (%)
44	=B2	=18000/D40	=A44*B44	=D40	=D44*C44/1000/1000	=100*(E44-E40)/E4
45						
46	Interpolated Output					
47	Interpolated Zero moment	first moment	Rel First moment	Retention time (hrs)	Pore Vol (L)	Mass Balance Error (%)
48	=Moment0(8,33,1,5)	=Moment1(8,33,1,5)	=B48/A48	=(C48-(B44/2))/60	=D48*60*D40/1000	=(B44-A48)/B44*10

Appendix S: Adjusted Column Retention Times, Pore Volumes, and Mass Balance Error

Modified Data

Column	Retention Time (hr)	Pore Volume (L)	Mass Balance Error (%)
1	10.7	17.0	-0.57
2	9.3	15.7	-0.54
3	10.4	17.5	6.69
4	12.3	19.3	-1.99
5	13.0	21.0	-5.95
6	12.4	20.6	-4.64
7	13.2	21.1	-2.26
8	11.9	19.1	-0.97

Appendix T: Gas Chromatograph Calibration Curves

Ret Time (min)	Concentration (ppm)	Area (count * s)
1.508	1	349.68
1.511	10	3864.22
1.509	100	39747.7
1.517	1000	393603

GC Calibration Curve, 11 Nov 03

$y = 0.0025x$
$R^2 = 1$
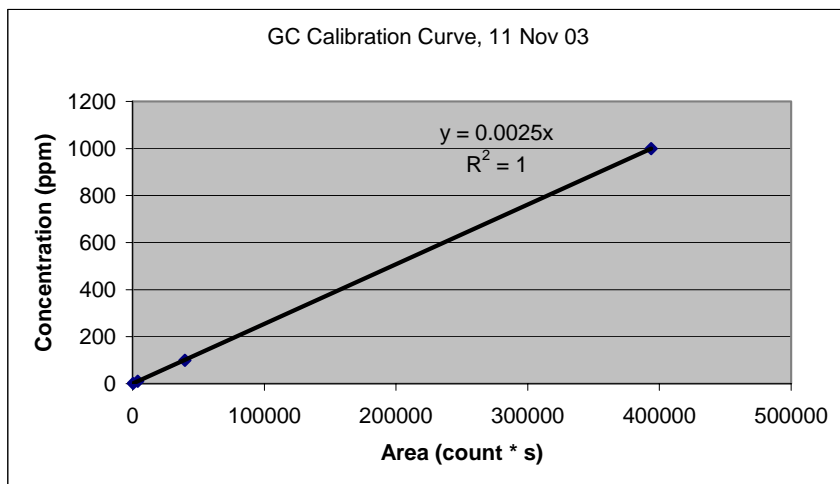
Ret Time (min)	Concentration (ppm)	Area (count * s)
1.513	1	360.87
1.512	10	3470.05
1.513	100	39419.3
1.515	1000	395949

GC Calibration Curve 2, 11 Nov 03

$y = 0.0025x$
$R^2 = 1$
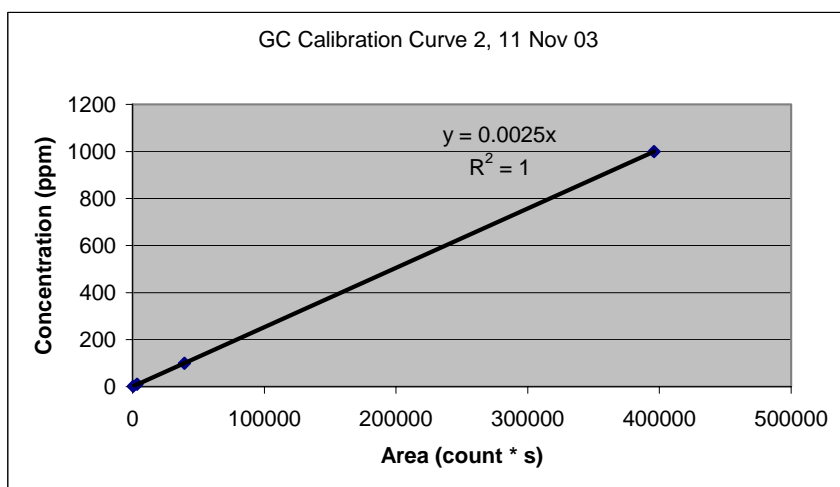
Ret Time (min)	Concentration (ppm)	Area (count * s)
1.601	1	717.58
1.599	10	3311.28
1.595	100	37135.3
1.596	1000	293110

GC Calibration Curve, 15 Nov 03
(Using Column 1 Effluent Water)

$y = 0.0034x$
$R^2 = 0.999$

Concentration (ppm) vs. Area (count * s)
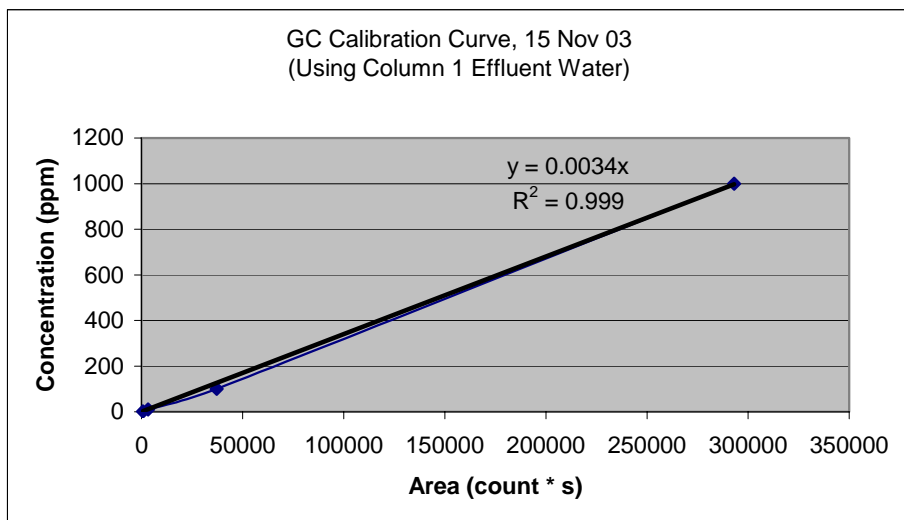
Ret Time (min)	Concentration (ppm)	Area (count * s)
1.597	1	592.62
1.596	10	3071.67
1.599	100	37100.6
1.599	1000	369265

GC Calibration Curve 2, 15 Nov 03
(Using Column 2 Effluent Water)

$y = 0.0027x$
$R^2 = 1$

Concentration (ppm) vs. Area (count * s)
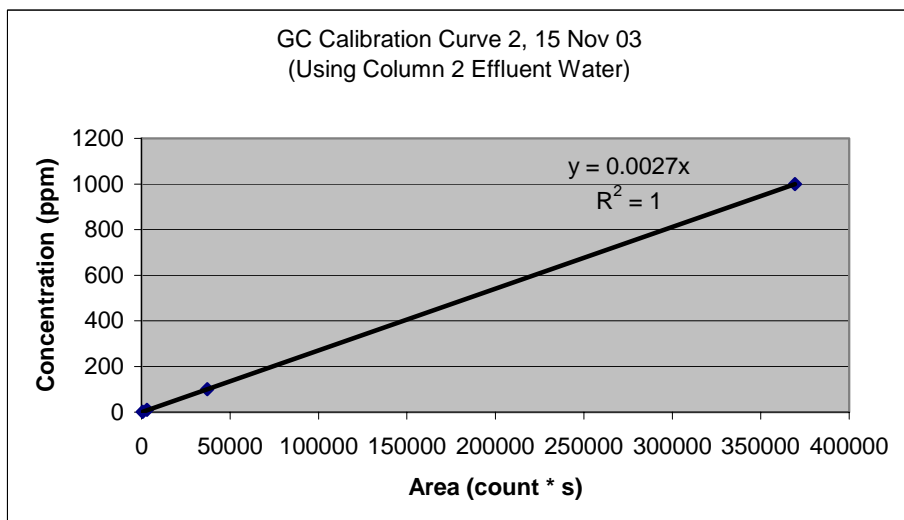
Ret Time (min)	Concentration (ppm)	Area (count * s)
1.592	1	437.91
1.591	10	3276.17
1.589	100	36471.2
1.593	1000	360254

GC Calibration Curve, 25 Nov 03

$y = 0.0028x$

$R^2 = 1$
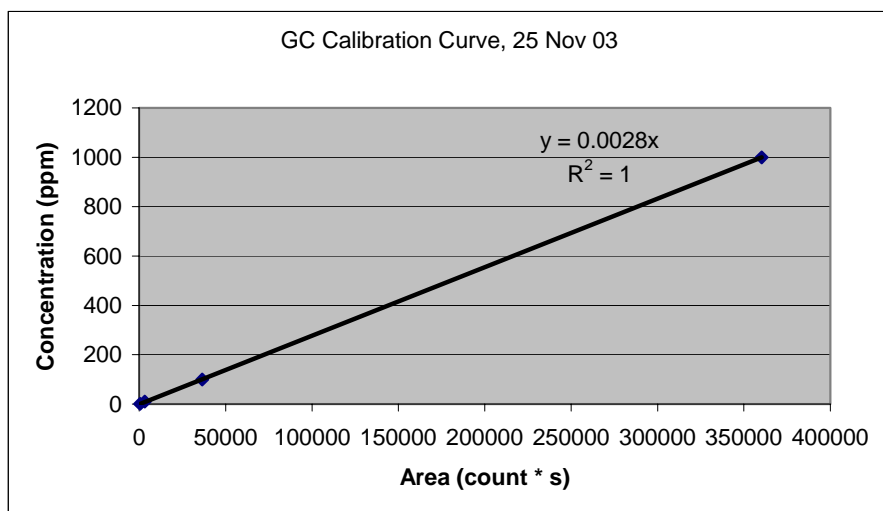
Ret Time (min)	Concentration (ppm)	Area (count * s)
1.601	1	351.8
1.6	10	3514.3
1.598	100	35673.1
1.598	1000	344253

GC Calibration Curve, 23 Jan 03

$y = 0.0029x$

$R^2 = 1$
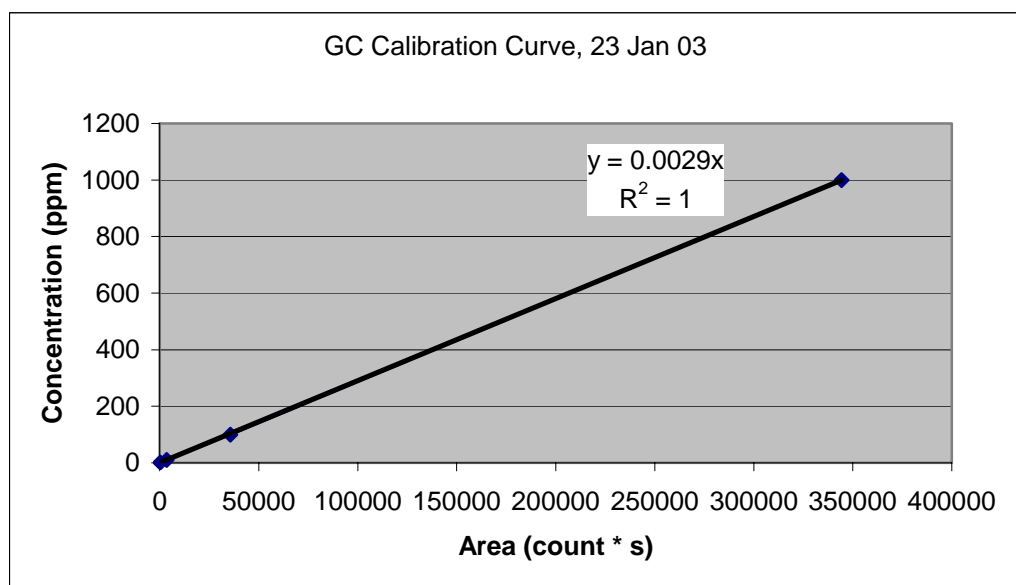
Appendix U: Gas Chromatograph Data

Influent Concentration (ppm)								
Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
25-Nov	21.05	396.16	315.59	0.00	416.74	1240.98	521.33	187.52
26-Nov	21.93	330.77	308.94	0.00	224.79	1118.17	265.53	229.82
29-Nov	10.66	1.67	0.00	0.00	943.65	749.47	0.72	424.29
30-Nov	4.31	688.66	0.00	0.00	1108.71	1027.13	456.97	714.80
1-Dec	927.42	1046.40	0.14	0.00	1096.34	846.06	1109.25	1.45
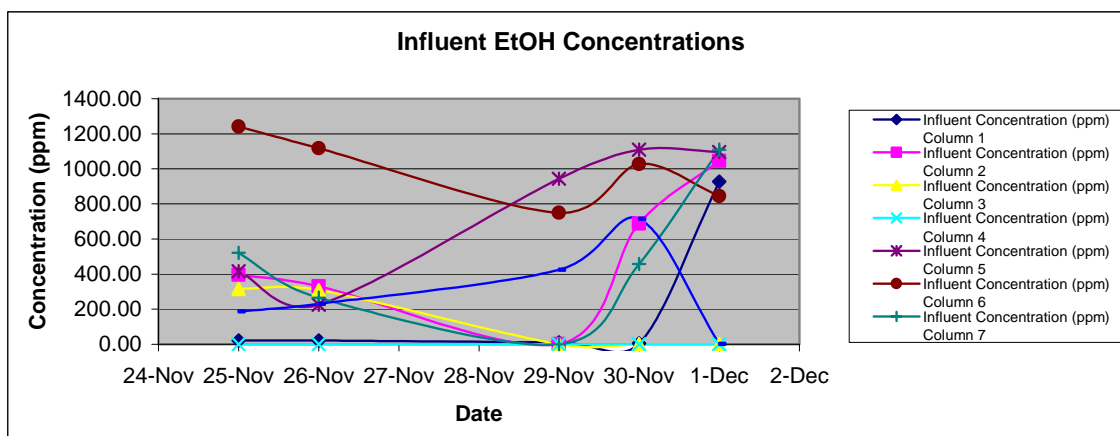
Effluent Concentration (ppm)								
Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
25-Nov	56.78	112.12	7.99	0.00	0.00	0.00	70.77	183.19
26-Nov	53.37	107.16	9.53	0.00	0.22	0.00	71.06	196.86
29-Nov	57.31	175.89	0.00	0.00	799.63	707.80	0.25	390.47
30-Nov	14.79	0.00	0.00	0.00	784.27	755.41	0.37	234.42
1-Dec	605.98	0.00	0.00	0.00	780.87	683.65	58.31	471.18
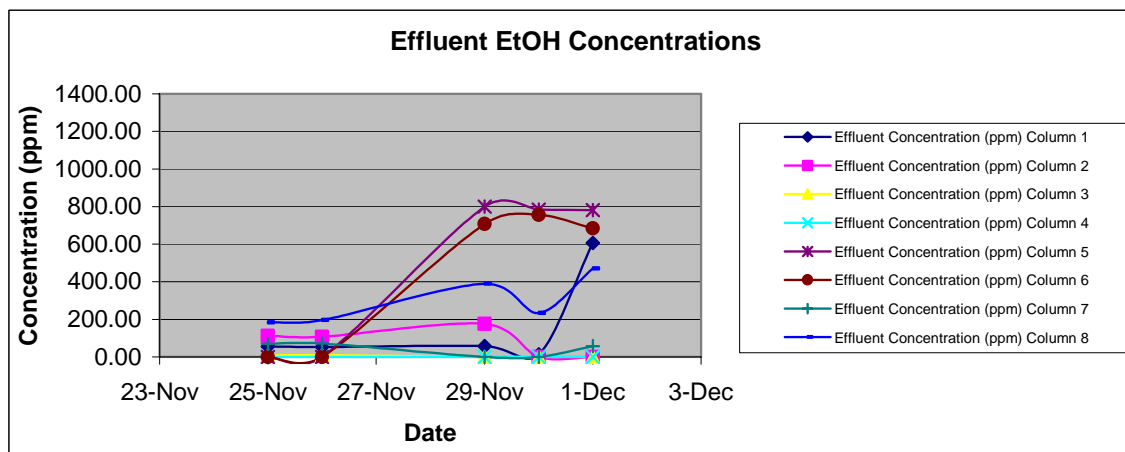
Appendix V: Gas Chromatograph Data after Modifications

Influent Concentration (ppm)								
Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
23-Jan	547.13	1836.63	41.29	1061.71	1369.89	1727.44	1516.37	1391.50
24-Jan	677.03	1415.13	2927.43	1343.35	1335.14	2262.45	0.00	1190.99
25-Jan	798.86	1695.11	668.51	562.68	1150.30	4287.10	1303.36	1373.63
27-Jan	1046.97	59.11	1606.13	1328.97	316.09	1194.50	1070.83	1484.20
29-Jan	198.41	116.44	0.00	386.09	473.97	9602.54	1083.00	14.13
30-Jan	231.90	127.66	1245.28	1155.24	1140.24	666.97	1924.01	92.90
31-Jan	1056.27	29.60	2542.99	155.82	7.47	2912.04	1373.70	70.53
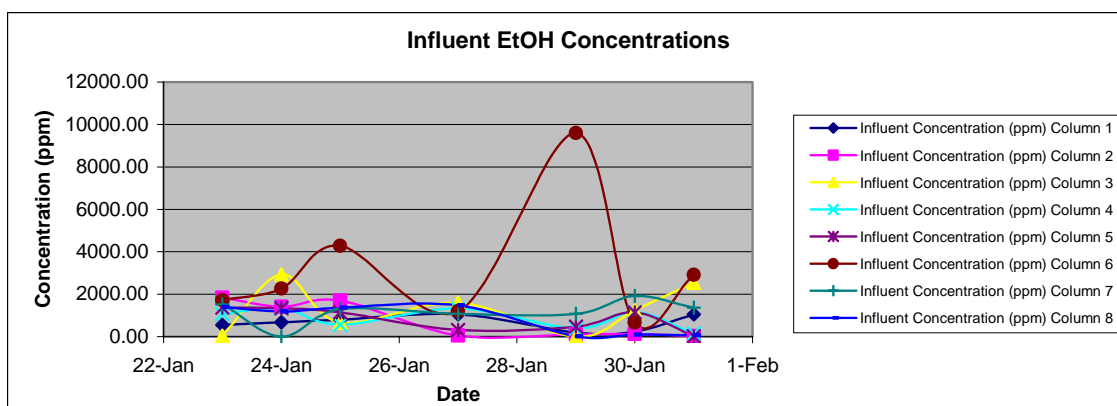
Effluent Concentration (ppm)								
Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
23-Jan	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24-Jan	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.00
25-Jan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27-Jan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29-Jan	0.00	0.00	0.00	0.00	0.00	165.44	0.00	0.00
30-Jan	0.00	0.00	0.00	0.00	0.00	210.62	0.51	0.00
31-Jan	0.00	0.00	0.00	0.00	0.00	173.63	0.51	0.00

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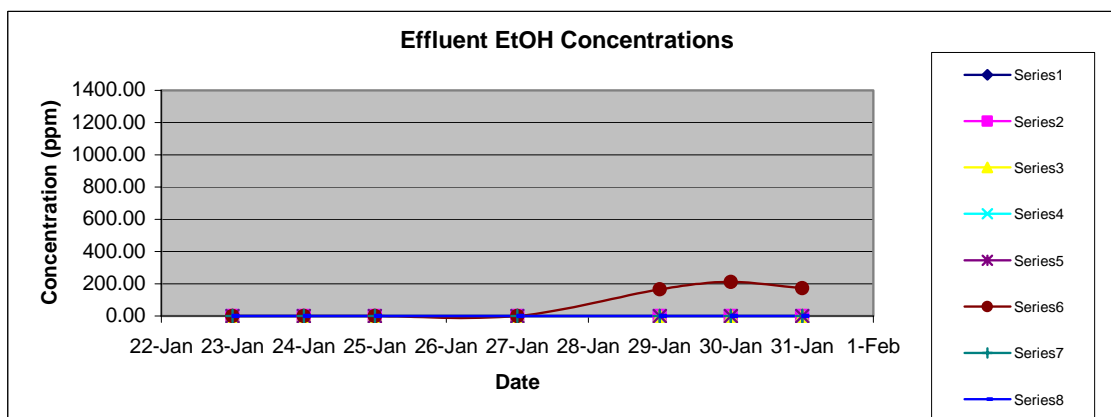
Appendix W: Oxygen Sensor Data
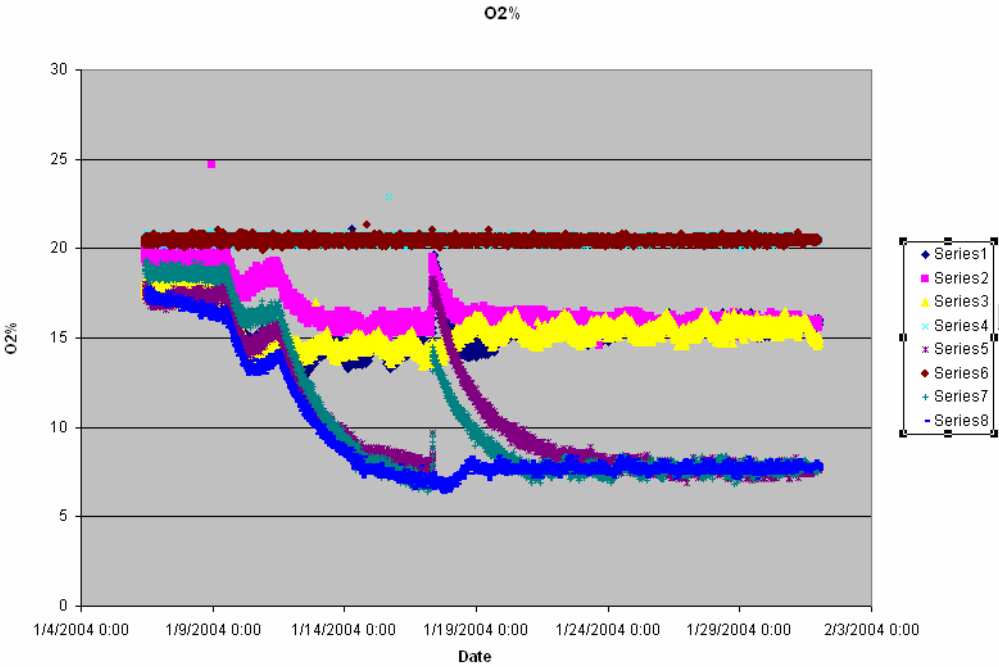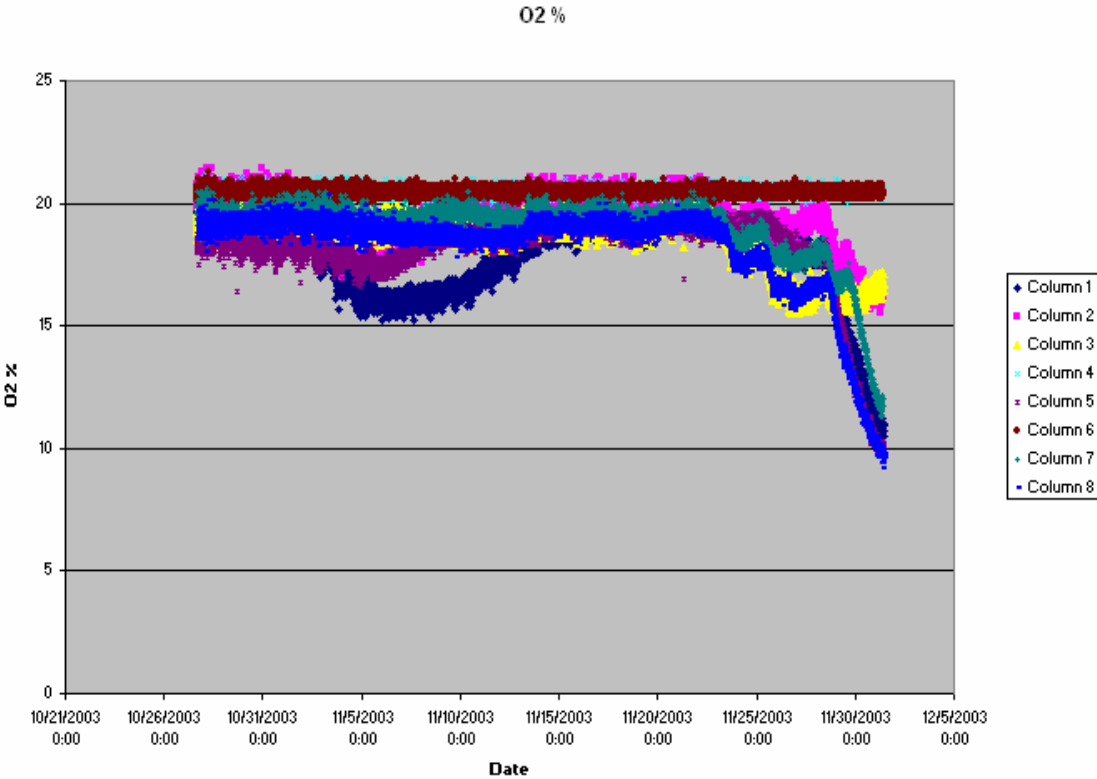
Appendix X: List of Materials

Equipment/Material	Manufacturer	Model Number	Description
Fixed Flow Pump Drive	Masterflex L/S	7543-30	Fixed speed peristaltic pump drive
Easy-Load II Pump Head	Masterflex L/S	77200-50	Peristaltic Pump head
Pump System	Masterflex C/L	77120-52	Variable speed peristaltic pump
DDL Air Compressor	GAST	6EBS	Air compressor
Oxygen Sensor	Japan Limited Battery Co. Ltd.	KE-50 F3	Oxygen sensor
Drying column with	W.A. Hammond	07193-00	Drier
TYGON Tubing	TYGON	3370IB	1/4 in. I.D.
Pump tubing	Masterflex	06404-17	Pump tubing
Pump tubing	Viton	06434-02	1/8 in. I.D. Pump tubing
Pump tubing	Viton	06434-01	1/16 in. I.D. Pump tubing
Pump tubing	Viton	07632-26	.89 mm I.D. Pump tubing
Tubing, Copper 1/8", 50 ft/rl	Cole-Parmer	34671-00	Copper tubing
Tubing, Copper 1/4", 50 ft/rl	McMaster-Car	UU-75190590	Copper tubing
Static mixer	Koflo	3/16-21	Static Mixer
Soil	Found on back side of airfield at WPAFB Area C		Sandy silt
Pipe, PVC, 8", 20 ft	Hughes Plumbing		PVC pipe
Tedlar gas sampling	Chemware	D1075012-10	On/Off, 8.1 L
Screw cap vials	Agilent	5182-0714	Screw cap vials, clear
Oxygen, conductivity, salinity, & temperature probe	YSI Inc	85/25 FT 99J0582 AA	YSI Conductivity Probe

Appendix Y: Example of Effluent Output Chromatogram

```
=================================================================
Injection Date  : 1/30/04 11:04:50 PM        Seq. Line :   11
Sample Name     : E3A                         Location : Vial 11
Acq. Operator   : KEVIN                             Inj :   1
                                              Inj Volume : 1 µl
Sequence File   : C:\HPCHEM\1\SEQUENCE\KMRUN1.S
Method          : C:\HPCHEM\1\METHODS\RICHSP.M
Last changed    : 1/23/04 9:39:33 PM by KEVIN
```
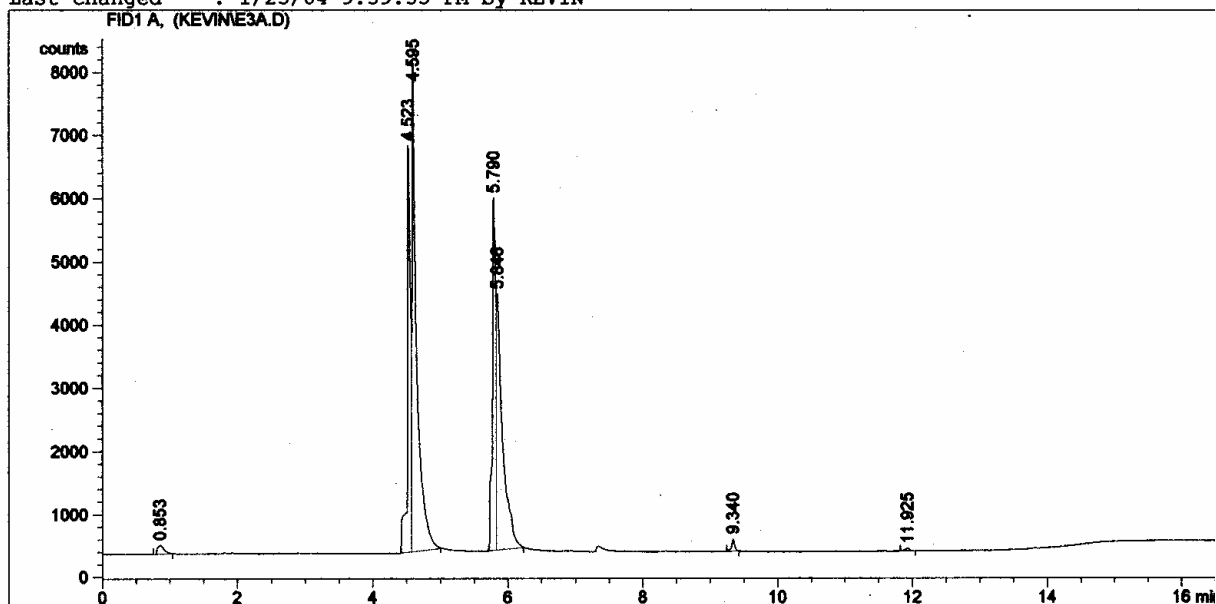


```
=================================================================
                      Area Percent Report
=================================================================

Sorted By           :      Signal
Multiplier          :      1.0000
Dilution            :      1.0000


Signal 1: FID1 A,

Peak RetTime Type  Width      Area       Height      Area
  #   [min]        [min]    counts*s    [counts]       %
----|-------|----|-------|-----------|-----------|---------|
   1   0.853 PB   0.0811   941.95282   138.94151   0.90244
   2   4.523 PV   0.0454  2.17850e4   6444.29736  20.87112
   3   4.595 VB   0.0608  3.64866e4   7768.58984  34.95586
   4   5.790 BV   0.0457  1.89926e4   5578.58398  18.19578
   5   5.846 VB   0.0808  2.53244e4   4059.73242  24.26200
   6   9.340 BB   0.0483   635.76074   188.91377   0.60909
   7  11.925 BP   0.0578   212.63667    45.25874   0.20372

Totals :                  1.04379e5   2.42243e4

 Results obtained with enhanced integrator!
=================================================================
                   *** End of Report ***
```
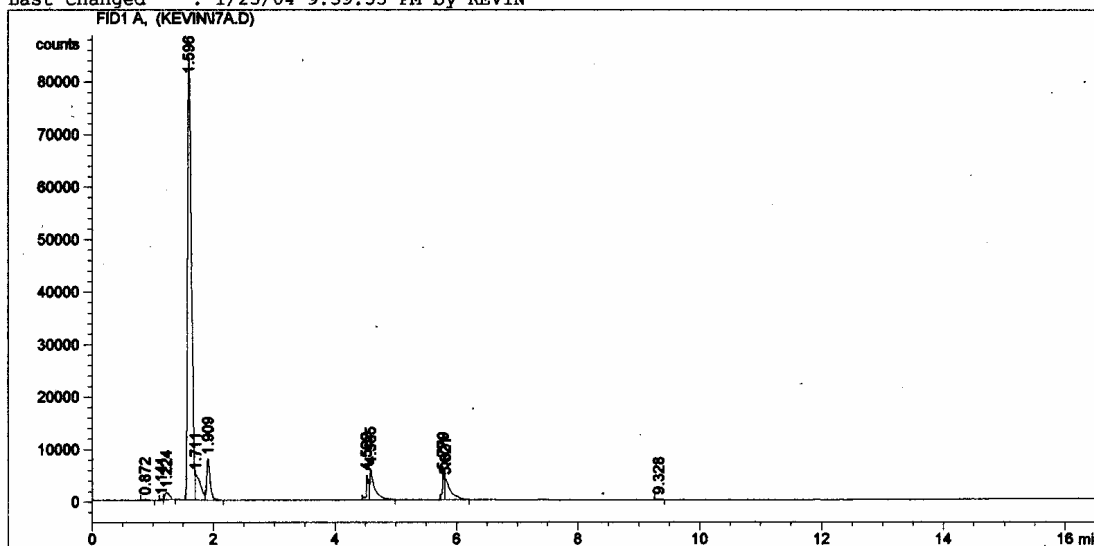
Appendix Z: Example of Influent Output Chromatogram

```
==================================================================================
Injection Date  : 1/30/04 10:13:50 AM          Seq. Line :    7
Sample Name     : I7A                           Location : Vial 7
Acq. Operator   : KEVIN                               Inj :    1
                                                Inj Volume : 1 µl
Sequence File   : C:\HPCHEM\1\SEQUENCE\KMRUN1.S
Method          : C:\HPCHEM\1\METHODS\RICHSP.M
Last changed    : 1/23/04 9:39:33 PM by KEVIN
```



```
==================================================================================
                        Area Percent Report
==================================================================================

Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000


Signal 1: FID1 A,

Peak RetTime Type  Width     Area       Height      Area
 #   [min]        [min]  counts*s    [counts]       %
----|-------|----|-------|----------|-----------|--------|
  1   0.872 PB    0.0745  690.60437   123.01650  0.13174
  2   1.141 PV    0.0362  529.36633   226.84450  0.10099
  3   1.224 VB    0.0760 7674.58496 1443.79712   1.46406
  4   1.596 PV    0.0677 3.73448e5   8.42438e4  71.24169
  5   1.711 VV    0.0768 2.90132e4   4848.47656  5.53478
  6   1.909 VB    0.0567 3.06917e4   7829.81250  5.85498
  7   4.522 PV    0.0358 1.31766e4   4832.42676  2.51367
  8   4.585 VB    0.0662 3.05932e4   5932.26611  5.83620
  9   5.779 PV    0.0389 1.32981e4   4868.44971  2.53684
 10   5.821 VB    0.0784 2.46825e4   3870.51196  4.70862
 11   9.328 BB    0.0481  400.61026   108.39806  0.07642

Totals :                 5.24198e5   1.18328e5

Results obtained with enhanced integrator!
==================================================================================
```

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Appendix AA: Method Detection Limit Data

x(bar) =	1.01 ppm

	x(i)	x(i)-x(bar)	(x(i)-x(bar))^2
1	0.87420	-0.13149	0.01729
2	0.90218	-0.10351	0.01071
3	1.22615	0.22046	0.04860
4	1.02022	0.01453	0.00021
Total	4.02274	0.00000	0.07682

SD =	0.160
t(.99) =	5.841

MDL =	0.93 ppm

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14. ABSTRACT
Groundwater contamination problems caused by methyl-tertiary butyl ether (MTBE) in subsurface waters have prompted the search for a gasoline oxygenate replacement. In order to avoid the problems encountered with MTBE, it is prudent to evaluate the fate and transport in the subsurface of proposed replacements, such as ethanol. In this study, ethanol transport and degradation in unsaturated soil was investigated using a series of eight soil columns. This preliminary study was to see if the soil column system components functioned properly, how similarly the eight soil columns performed, and if soil oxygen concentration affected degradation of ethanol. Tracer tests, using sodium chloride, determined the hydraulic characteristics of the soil columns. Oxygen sensors measured microbial activity in the soil columns when ethanol was added to the columns. The sensors were part of a control system that stabilized oxygen concentration at two levels (8% in four columns and 16% in four columns) to see the effect of oxygen concentration on ethanol degradation. A gas chromatograph (GC) was used to quantify column influent and effluent ethanol concentrations.

The tracer tests showed an average retention time, pore volume, and mass balance error of 13.3 hr (+/- 1.4), 18.9 L (+/- 2.0), and 1.3% (+/- 3.8), respectively. The oxygen sensor data, which indicated a drop in oxygen concentration over time when ethanol was added, suggested that microbial activity was occurring. The microbial aerobic metabolism of ethanol caused the oxygen concentrations to drop to the set points of 8% and 16%, at which they stabilized. The GC analysis also showed ethanol degradation. Influent ethanol concentrations were ~ 1000 ppm, column effluent concentrations were at or near the method detection limit (MDL) of 1 ppm for both oxygen concentrations.

The soil columns, constructed as part of this research, were demonstrated to be a good laboratory system that could be used to study aerobic degradation of ethanol in the vadose zone. Further research is required to test other fuel oxygenates to see if they will degrade in this system. Such oxygenate degradation studies will be critical in helping to find a safe alternative to MTBE.

15. SUBJECT TERMS
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